The Journal of
Allergy
VOL. 30, NO. 5
SEPTEMBER—OCTOBER, 1959

ORIGINAL ARTICLES

STUDIES ON THE ALLERGENICITY OF CORN PRODUCTS

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With the Technical Assistance of Joan Best

HYPERSENSITIVITY to corn (maize) is a well-defined clinical entity, but there is no general agreement as to its incidence in allergic persons. Published estimates of its occurrence in patients with food allergy vary from 0.16 per cent to 30 per cent.

Likewise, there is no agreement as to whether sugars derived from the hydrolysis of corn have allergenic properties. Ratner and Gruehl,[1] using the technique of guinea pig anaphylaxis, found no evidence of antigenicity in samples of dextrimaltose mixtures, corn syrup, or cyrstalline sugars derived from corn. They also showed that while the malt extracts and barley malt used in the preparation of certain forms of dextrimaltose preparations are highly anaphylactogenic, there was no evidence that antigenic amounts of these substances were present in the final product.

On the other hand, Rinkel, Randolph, and Zeller,[2] and Randolph and Yeager[3] found evidence, by clinical trial, of allergy to corn and sugars and starches derived from corn in approximately 25 per cent of patients with food allergy, and they rank corn second only to wheat as a food allergen. These observations have been questioned by Loveless[4] and Bernton[5] who, using a masked ingestion test, were unable to confirm these findings.

Since various types of corn sugars are used widely in infant feeding as carbohydrate modifiers of all types of milk formulas, especially during the period when gastrointestinal disturbance (colic) is common, there has

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This study was supported by a grant from the Borden Company, New York, New York.
Received for publication Dec. 26, 1958.

been raised the question of whether the sugars could be a factor (in addition to milk) in the production of these symptoms on an allergic basis. As an extension of the work of Ratner and Gruehl,[1] we decided to test four such sugars for possible antigenicity, using more sensitive immunologic techniques, that is, the passive transfer reaction and the Schultz-Dale test.

The sugars studied were as follows:

1. A maltodextrin preparation formed by the acid hydrolysis of cornstarch (dextrose equivalent 24).

2. A commercial edible grade of dextrose, also prepared by the acid hydrolysis of cornstarch.

3. Sucrose, derived from cane sugar, used as a control.

4. A dextrimaltose preparation prepared by the enzymatic action of barley malt on cornstarch (dextrose equivalent 36).

5. A corn syrup prepared by the acid hydrolysis of cornstarch (dextrose equivalent 38).

These sugars are designated by number throughout the experimental work.

PASSIVE TRANSFER TESTS

Serum was obtained from two known corn-sensitive persons. Neither was sensitive to barley, and the sera of both gave consistently positive reactions to whole corn extract by passive transfer test. These full-strength sera were prepared in dilutions of 1:2, 1:5, 1:10, 1:100, and 1:1,000, and all were used in preparing suitable testing sites on a nonallergic recipient.

Six separate sets of sites were prepared, beginning with the full-strength serum and continuing down to the 1:1,000 dilution. The first set was tested with whole corn extract (1,000 PNU per cubic centimeter) both to prove the presence of corn reagin and to quantitate its strength by the dilution technique. Each of the other five sets of sites was tested with a 10 per cent aqueous solution of one of the five sugars described. A positive passive transfer reaction was considered to be present when a 2 plus reaction (a wheal measuring 0.5 to 1.0 cm. in diameter) was obtained and the control was negative.

A total of nine passive transfer studies were performed. In each instance the serum showed a positive response to testing with whole corn extract as far as the 1:10 or 1:100 dilutions, while all controls were negative. Sugar No. 4 gave a positive reaction in four of the nine passive transfer tests in dilutions of 1:2 or 1:5. Sugar No. 5 gave three positive tests in nine trials, while the other three sugars, including the sucrose control, gave no significant responses.

A 25 per cent concentration of the sugars was used in two additional passive transfer tests but gave positive reactions not only in all the test sites but in the control areas as well. These were deemed to be irritant rather than allergic reactions.

SCHULTZ-DALE STUDIES

Virgin female guinea pigs, weighing approximately 300 grams each, were sensitized with a fresh corn extract potentiated by the use of Freund's adjuvant. These animals were derived from stock which had been on a diet free of corn and barley for more than four generations. The guinea pigs were

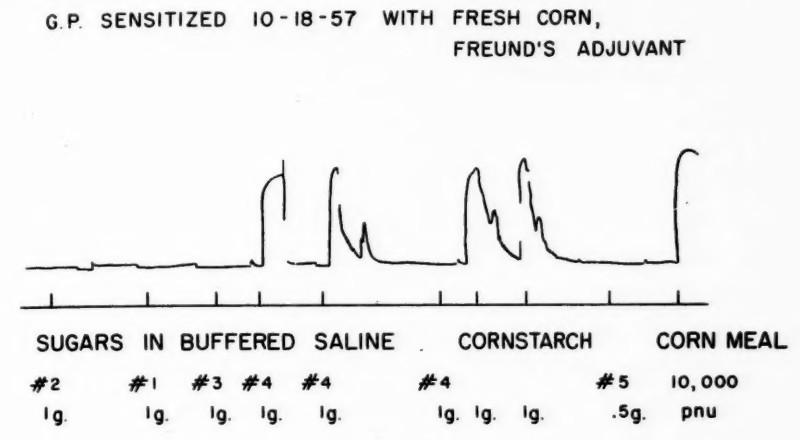

Fig. 1.

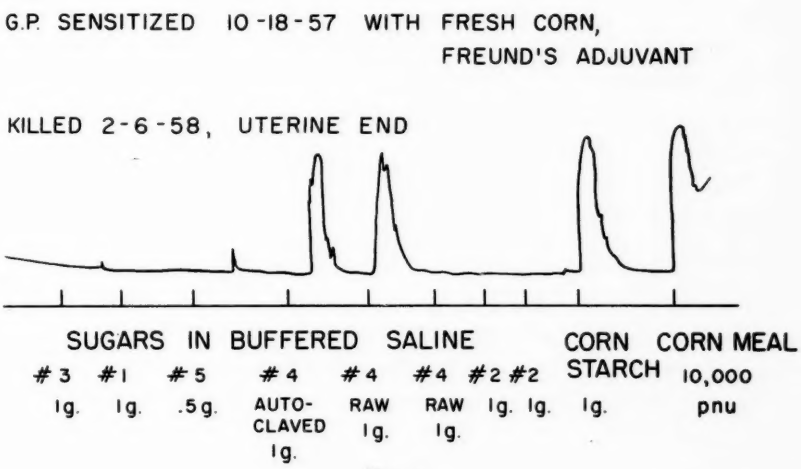

Fig. 2.

Figs. 1 to 6.—Demonstration of a fraction of corn antigen in one sugar derived from cornstarch. Kymograms of the reactions of the uterine horns of sensitized guinea pigs following stimulation with the designated materials in the Schultz-Dale bath. (All guinea pigs were sensitized to fresh whole corn extract potentiated with Freund's adjuvant.)

Figs. 1 and 2 show a contraction of the uterine horn to sugar No. 4, raw or autoclaved. When sugar No. 4 no longer reacts, corn meal still causes a reaction, demonstrating that the antigenic property of sugar No. 4 is only a fraction of the whole corn antigen. (The cornstarch response is a known nonspecific reaction.)

sacrificed after two months, and strips of the uterine musculature were tested in the Schultz-Dale muscle bath as follows:

Group I.—The uterine horns were first shocked with corn meal (10,000 PNU) until contractions were no longer obtained, and then they were tested to the various sugars. Sample tracings are shown in Figs. 5 and 6. In none

of these tests did any of the sugars produce any response after exhaustive shocking with corn meal, but these horns were still able to respond to either cornstarch or histamine.

Group II.—In this group the uterine horns were challenged with the various sugars in random order before being challenged with corn meal extract. Figs. 1, 2, 3, and 4 indicate the varied responses obtained. In approximately one-half (eleven) of the horns tested, sugar No. 4 gave a definite positive reaction, as shown in tracings 1 and 2. In the other twelve tests no reactions could be obtained to any of the sugars, as shown in tracings 3 and 4. Autoclaving of sugar No. 4 had no apparent effect on its ability

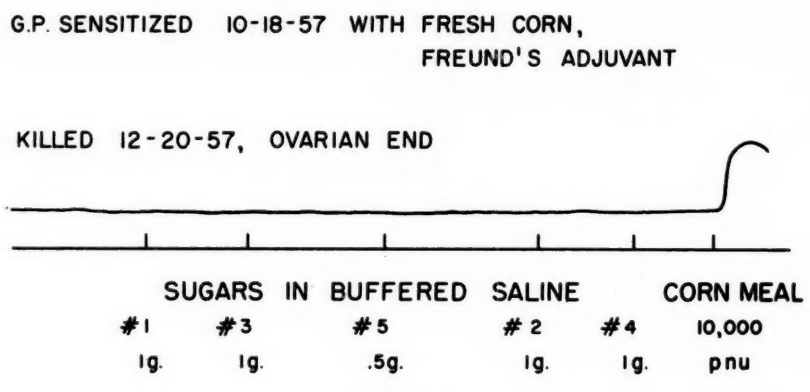
G.P. SENSITIZED 10-18-57 WITH FRESH CORN, FREUND'S ADJUVANT
KILLED 12-20-57, OVARIAN END
SUGARS IN BUFFERED SALINE
CORN MEAL
#1 #3 #5 #2 #4 10,000
1g. 1g. .5g. 1g. 1g. pnu

Fig. 3.

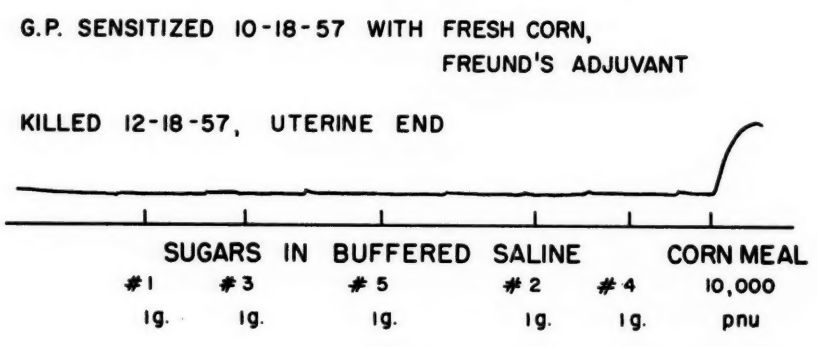
G.P. SENSITIZED 10-18-57 WITH FRESH CORN, FREUND'S ADJUVANT
KILLED 12-18-57, UTERINE END
SUGARS IN BUFFERED SALINE
CORN MEAL
#1 #3 #5 #2 #4 10,000
1g. 1g. 1g. 1g. 1g. pnu

Fig. 4.

Figs. 3 and 4 show no contraction when the uterine horn is stimulated by any of the sugars. This was true in half the animals tested.[12]

to produce a contraction in the sensitized muscle. It will be noted in Figs. 1 and 2 that after sugar No. 4 failed to produce any additional response in the uterine horn, a positive response could still be obtained to corn meal, cornstarch, and histamine. It should also be noted that cornstarch caused a reaction in every instance in which it was used, even after the muscle strip had been made nonreactive to corn meal. It has been shown that cornstarch gives a nonspecific reaction in any uterine horn, regardless of the presence or absence of sensitization to corn.

INTERPRETATION OF RESULTS

The passive transfer reaction, considered by many to be a more sensitive immunologic test than gross anaphylaxis of the Schultz-Dale test, gave definite positive reactions to sugars No. 4 and No. 5 in nearly one-half the tests performed, while no reaction was obtained to the other sugars. This would indicate that sugars No. 4 and No. 5 contain sufficient amounts of corn antigen to be irregularly identified by the passive transfer technique. It is possible, however, that we are dealing with an irritant factor which might be eliminated with further dilution of the testing materials, as was suggested by the results obtained with 25 per cent concentrations of the sugars.

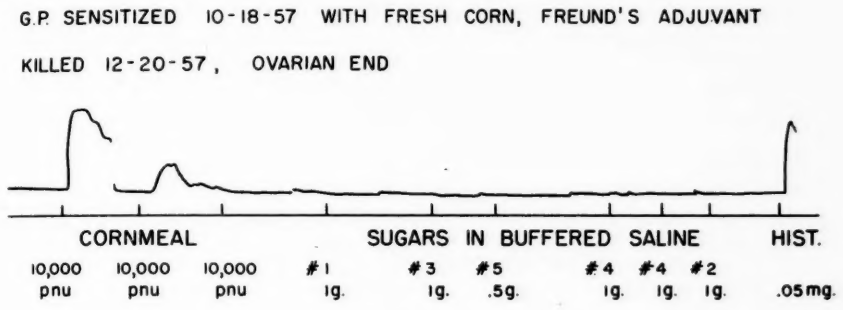

Fig. 5.

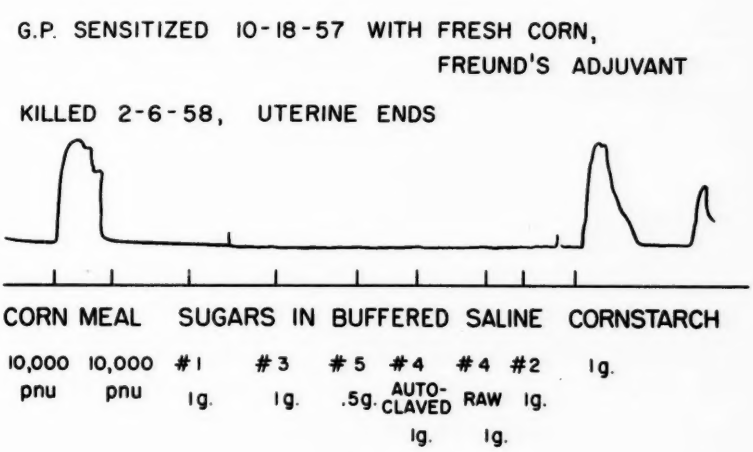

Fig. 6.

Figs. 5 and 6 demonstrate the exhaustion of whole corn antibody by stimulation with corn meal, so that sugar No. 4 does not react when it follows corn meal. Fig. 6 confirms the nonspecific nature of the cornstarch reaction occurring after the muscle has been depleted of corn antibody.

In the Schultz-Dale muscle bath, sugar No. 4 produced muscle contractions in several instances but never depleted corn antibody, since corn meal always produced a reaction after sugar No. 4 had failed. This is taken to indicate that the amount of corn antigen in sugar No. 4 is small and cannot

properly exhaust the uterine strip, which will still respond to large doses of the antigen (law of multiple proportions), or possibly that sugar No. 4 contains only one or a few of the antigens present in corn extract.

SUMMARY AND CONCLUSIONS

1. Five sugars of the type used in infant feeding have been tested for the presence of corn antigen by the passive transfer and Schultz-Dale techniques. Sugar No. 1 is a maltodextrin preparation, and sugar No. 2 is dextrose, both prepared by acid hydrolysis of cornstarch. Sugar No. 3, used as a control, is sucrose (cane sugar). Sugar No. 4 is a dextrimaltose prepared by the enzymatic action of barley malt on cornstarch, while sugar No. 5 is a commercial corn syrup prepared by the acid hydrolysis of cornstarch.

2. By the passive transfer and Schultz-Dale techniques, sugars No. 1, No. 2, and No. 3 have no demonstrable antigenic activity.

3. By passive transfer, sugars No. 4 and No. 5 show inconstant evidence of the presence of corn antigen.

4. By the Schultz-Dale technique, sugar No. 4 produced positive responses in approximately one-half the animals tested. These positive reactions may be due to the presence of small amounts of whole corn extract or to the presence of one or more antigens in the extract.

5. From the data presented, there is suggestive evidence that detectable amounts of corn antigen or antigens may be present in certain sugars used in infant feedings. No evidence is presented to indicate that these amounts are sufficient to be important; rather, it is suggested that additional work, both experimental and clinical, is indicated to clarify the role of corn as a possible allergen in infant feeding.

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THE USE OF ADJUVANTS IN SENSITIZATION OF THE MOUSE

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WE HAVE repeatedly observed[1] in experiments on mice that a consequence of the administration of *Hemophilus pertussis* phase I organisms given in conjunction with a protein antigen is the enhancement of anaphylactic sensitization to the foreign protein antigen. A concomitant increase in the sensitivity of the pertussis-inoculated mouse to serotonin and histamine results also.[2-4] The former effect is probably related to an increase in antibody titer, fixed or sessile, even though neither circulating precipitating nor univalent antibodies have been detected.[1] Since the use of extraneous substances, including bacteria, for the intensification of antibody responses is well known in other species,[5] it was deemed of interest to investigate the adjuvant effect of a variety of agents in the mouse primarily to correlate anaphylactic sensitization with an increase in antibody titer and a decrease in resistance to the lethal effects of histamine.

MATERIALS

Mice.—Female white mice of the Swiss-Webster strain, each weighing about 20 grams at the time of the experiments, were obtained from Tumblebrook Farm and were used exclusively.

H. pertussis.—A concentrated paste of phase I organisms containing Merthiolate 1:1,000 was supplied by Merck Sharp & Dohme, Inc. This was washed and diluted as required. The semisynthetic, cell-free culture filtrate of Kuwajima and associates[6] on which *H. pertussis* had been grown was used in certain of the experiments in the place of *H. pertussis* vaccine.* This was dialyzed and concentrated.

Adjuvants.—The following adjuvants were employed: *India ink,* commercial Higgins American India Ink, black; *gelatin* (Bacto-Gelatin, Difco), 5 per cent solution in 0.85 per cent saline; *agar* (Bacto-Agar, Difco), 0.25 per cent solution in 0.85 per cent saline; *kaolin* (5 per cent suspension of Infusorial Earth, B & A), in 0.85 per cent saline; *dextran* (Plavolex, Wyeth), 6 per cent

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This investigation was supported by a research grant from the National Institutes of Health, United States Public Health Service.

Received for publication Jan. 28, 1959.

*We are grateful to Miss Wetterlow of the Public Health Department, Institute of Laboratories, Jamaica Plain, Massachusetts, for making this filtrate available to us.

w/v; *polyvinylpyrrolidone (PVP)* (20 per cent solution of Plasdone, General Aniline and Film Corp.), in 0.85 per cent saline, diluted as needed to make a 10 per cent solution; *zymosan,* concentrations ranging from 0.2 to 1.0 per cent suspension in 0.85 per cent saline. The zymosan had been either prepared in this laboratory according to the method of Pillemer and Ecker[7] or purchased from Standard Brands.

METHODS

Sensitization.—Sensitization was effected by a single intraperitoneal injection of a mixture of a given antigen (either 0.03 ml. of horse serum or 1.0 mg. of thrice-recrystallized ovalbumin) and the appropriate "schlepper" or adjuvant agent in a 0.5 ml. volume.

TABLE I. EFFECT OF ADJUVANT ON HISTAMINE AND ANAPHYLACTIC SENSITIZATION

ADJUVANT*	SENSITIZING ANTIGEN†	HISTAMINE PHOSPHATE‡	CHALLENGE ANTIGEN§	
			HORSE SERUM	OVALBUMIN
—	—	1/10‖	0/11	
—	Ea	0/10		1/10
—	Ea (I.V.)			0/20
—	HS	3/19	5/35	
H. pertussis	Ea	24/25		16/16
6×10^9 cells	HS	10/10	52/52	
Filtrate concentrate	HS	5/6	9/9	
	Ea			9/10
India ink	Ea	0/19		10/10
	HS		19/19	
Gelatin 5%	Ea	3/19		1/10
	HS		3/15	
Agar 0.25%	Ea	2/19		0/10
	HS		8/17	
Kaolin 5%	Ea	2/18		10/10
	HS		13/15	
Kaolin 5%	Ea (I.V.)			12/12
Dextran 6%	HS	1/19	8/18	
PVP 10%	Ea	3/19		2/10
20%	HS		4/16	
Zymosan 1 mg.	HS		9/17	
3 mg.	HS	0/20	27/40	
5 mg.	HS		27/44	
3 mg.	—	1/10	0/10	

*Contained in 0.5 ml. volume.
†0.03 ml. horse serum (HS) or 1 mg. ovalbumin (Ea) given intraperitoneally except where otherwise indicated.
‡0.03 mg. per gram mouse of 0.2 per cent solution given intraperitoneally.
§0.1 ml. HS or 1 mg. Ea in 0.1 ml. 0.85 per cent saline given intravenously.
‖Number of animals dead per number of animals challenged.

Histamine Sensitivity.—On the fourth day after sensitization the mice received 0.03 mg. of histamine phosphate per gram of animal intraperitoneally as a 0.2 per cent solution. This is approximately the LD_{100} dose for pertussis-inoculated mice. The results of the challenge are recorded as number of animals dead per number of animals injected.

Anaphylactic Sensitivity.—On the tenth day after sensitization mice were challenged by the intravenous injection of the homologous antigen, 0.1 ml. of horse serum or 1 mg. of ovalbumin in 0.1 ml. saline. The results are recorded as number of animals dead per number of animals challenged.

RESULTS

As can be seen from Table I, only *H. pertussis* was found capable of decreasing the resistance of the white mouse to histamine. On the other hand, all of the adjuvants were found capable of enhancing sensitization to some varying extent. The particulate substances—India ink, kaolin, and zymosan—engendered the highest degree of sensitization, and the more soluble agents produced the least. These findings are quite compatible with the concept of enhancing antibody formation by particulate matter onto which soluble antigen has been adsorbed.

TABLE II. TIME RELATIONSHIP BETWEEN ZYMOSAN AND ANTIGEN ADMINISTRATION AND ANAPHYLACTIC SENSITIZATION

DAY OF ADMINISTRATION											
-6	-4	-2	0	2	4	6	8	10	12	14	16
HS*			Z†		1/20‡			1/16			
	HS		Z			0/20		0/19			
		HS	Z				1/20	0/20			
			Z HS					24/40			
			Z	HS				7/20	7/20		
			Z		HS			4/16		6/17	
			Z			HS		0/18			8/16
			Z I.V. HS I.P.					6/18			
			Z		Z	HS I.P.		Day 11 0/20		5/20	
			Z					0/10			

*Sensitization by 0.03 ml. horse serum (HS) given intraperitoneally.
†3 mg. zymosan given intraperitoneally in 0.5 ml. volume.
‡Intravenous challenge by 0.1 ml. horse serum; number of animals dead per number of animals challenged.

The optimal time relationship for the administration of antigen and adjuvant resulting in maximal sensitization was studied by means of the zymosan system. Antigen (horse serum) was given intraperitoneally to mice at various time intervals before and after the intraperitoneal inoculation of 3 mg. of zymosan as well as concurrently. About half of the mice of each time group were challenged by the intravenous injection of 0.1 ml. of antigen on the tenth day following the initial inoculation of the sensitizing antigen. The other half of the group of mice in each series were challenged on the tenth day following the inoculation with zymosan. Table II shows the results, given as number of animals dead from anaphylactic shock per number of mice challenged. Sensitization is seen to be best accomplished by administering the antigen together with the zymosan. Antigen given prior to zymosan does not enhance sensitization; no increase in the number of anaphylactic deaths

is noted with administration of the challenge dose either ten days after the sensitizing dose or ten days after the zymosan, even when the sensitizing dose precedes the zymosan by only two days. Antigen given up to six days after the administration of zymosan results in a relatively high degree of sensitization when the challenge dose is given ten days subsequently. As the interval between the sensitizing and challenge dose is decreased, the number of anaphylactic deaths decreases. Within the limits of the experiment, it can be said that the minimal time interval between the sensitizing and challenge doses for maximal anaphylactic response is ten days. The relationship between the time of zymosan administration and sensitizing antigen administration does not appear to be critical, provided that the latter is given concurrently or following the adjuvant. The studies further revealed that the intravenous administration of zymosan does not engender a higher degree of sensitivity and that repeated inoculations of zymosan have no greater effect in enhancing sensitization.

TABLE III. ANTIBODY TITRATION OF MOUSE ANTISERUM BY BOYDEN TECHNIQUE

SERUM DILUTION	DAY ON WHICH HORSE SERUM GIVEN IN RELATION TO ZYMOSAN							ZYMOSAN I.V. HORSE SERUM I.P. BOTH ON DAY 0
	-6	-4	-2	0	2	4	6	
1:5	–	–	–	++	++	–	–	++
1:10	–	–	–	++	++	–	–	++
1:20	–	–	–	++	++	–	–	++
1:40	–	–	–	++	++	–	–	++
1:80	–	–	–	++	++	–	–	++
1:160	–	–	–	++	++	–	–	±
1:320	–	–	–	–	++	–	–	±
1:640	–	–	–	–	±	–	–	±
1:1,280	–	–	–	–	±	–	–	–
1:2,560	–	–	–	–	±	–	–	–
Control, uncoated cells	–	–	–	–	–	–	–	–
Anaphylactic deaths	1/16	0/19	0/20	10/20	7/20	4/16	0/18	6/18

Sera withdrawn on tenth day after intraperitoneal zymosan, prior to challenge.
Reactions: ++, positive; ±, questionable; –, negative.

The specific effect of zymosan on the titer of antibody was further studied because of the relationship of zymosan to the immune system[8] and the above observations that it possesses the marked ability to enhance sensitization. Blood was withdrawn from the ophthalmic sinus ten days after zymosan administration, just prior to challenge. Blood samples from mice within each group were pooled and allowed to clot, and the expressed serum was removed. Antibody studies were carried out by the Boyden tanned-red-cell technique,[9] using human red blood cells of type A in the titrations. The results of these studies are seen in Table III. A direct correlation is observed between maximal anaphylactic sensitization and highest antibody titer. However, anaphylactic deaths (25 per cent) did occur in one group in the absence of any titrable antibody.

DISCUSSION

Anaphylactic sensitization by the use of *H. pertussis* may be attributed to the adjuvant capabilities of bacteria in general. They have this capacity in common with a variety of extraneous and unrelated substances as foreign proteins, lipids, carbohydrates, metallic salts, inert adsorbents, etc. One of the criteria of a classical adjuvant is demonstrated by *H. pertussis* in that adsorption of a soluble antigen may take place on its cellular surface, thus allowing for increased retention of antigen in the tissues and providing for a slower and more gradual release of antigen for prolonged stimulation of antibody-forming sites. In addition, there is the possibility of wider dissemination of the antigen in the body with its inevitable greater accessibility to the antibody-forming sites as a result of phagocytosis of particulate matter by the cells of the reticuloendothelial system.

Most of the experimental data, however, seem to mitigate against the acceptance of the classical definition of adjuvant as applied to the mode of *H. pertussis* action. Previous studies[1] utilizing Freund's adjuvant in the white mouse have shown that although anaphylactic sensitization does occur, it neither appears as rapidly nor reaches the high degree of sensitization as it does with *H. pertussis.* Adler[10] has shown appreciable titers of antibody to antigens in the mouse, using an incomplete Freund's adjuvant at a time (twenty-eight days) beyond which maximum anaphylactic sensitization is known to occur (ten to fifteen days). Freund's adjuvant does not enhance histamine sensitivity.[11]

In addition, there are the following facts: (1) *H. pertussis* also increases susceptibility to histamine[2] and serotonin[3, 4] in the mouse. (2) *H. pertussis* in various stages of growth other than phase I does not have the ability to sensitize.[12] (3) Heating *H. pertussis* causes a marked decrease in the capacity of the organism to sensitize.[13, 14] (4) Other serologically related organisms, such as *H. influenzae,* fail to enhance either anaphylactic sensitization or susceptibility to histamine.[15]

The results of this study indicate that the typical adjuvants which have been used previously to increase antibody titer in other species are capable of enhancing anaphylactic sensitization in the mouse. The zymosan studies indicate that an increased antibody titer is apparently responsible for this sensitization. However, anaphylactic deaths were noted even though circulating precipitins could not be detected in all the sera by the tanned-red-cell technique of Boyden. It is to be recalled that precipitins have not been detected previously by either the ring technique or the Heidelberger-Kendall quantitative procedure in the sera of mice made highly sensitive.[1] This could mean that only very small, immeasurable quantities of circulating antibody need be available for anaphylaxis to occur. Passive transfer studies[16, 17] in pertussis-inoculated mice have indicated that about 4 to 8 mg. of antibody nitrogen per kilogram of the mouse's weight would consistently sensitize the animal. If this remained as circulating antibody, it would be well within the limitations of the Boyden titration technique. Another possibility is that the

type of sensitizing antibody produced cannot be measured by the techniques employed. Our previous studies[18] have suggested that *H. pertussis* is capable of enhancing the production of more than one type of antibody, depending upon the antigen with which it is administered. Thus, it was found that there were produced in the mouse a sensitizing antibody against diphtheria toxoid and a protective antibody in response to stimulation by tetanus toxoid.[19] A final proposition is that fixed or tissue antibody is primarily responsible for anaphylaxis. This concept is of considerable interest and will bear extensive investigation.

The mechanism for the enhancement of anaphylactic sensitization by *H. pertussis* is not understood, but there is a tendency to relate this phenomenon to the concomitant sensitization of the pertussis-inoculated mouse to histamine and serotonin,[12] a mechanism which also is not known. None of the adjuvant agents used in these experiments had any effect upon sensitization to histamine. In this the action of *H. pertussis* as a "schlepper" is unique in that both anaphylactic sensitization and sensitivity to histamine and serotonin are enhanced. This speaks for a common mechanism for both phenomena rather than an adjuvant action for anaphylactic sensitization with a distinct mechanism for sensitization to histamine. A recent review[12] discusses the available evidence for a common pathway, but experimental data at present are not sufficient to support any concrete theory.

Studies[20] in the mouse comparable with those of Condie and associates[21, 22] and Johnson and colleagues,[23] who noted that endotoxin given together with bovine serum albumin to the rabbit enhances antibody production against the foreign protein, have given an impetus to the question of mechanism. The ultimate experiment for the elucidation of the "adjuvant" effect of *H. pertussis* probably lies in an understanding of the effect of its endotoxin on the cellular system responsible for antibody production, even as Condie and associates[21] have conjectured for meningococcal endotoxin. Experiments along these lines are currently in progress and will be the subject of a subsequent publication.

SUMMARY

Particulate adjuvants, such as India ink, kaolin, and zymosan, were capable of enhancing anaphylactic sensitization in the mouse to a foreign protein antigen. Sensitization by the above was as pronounced as with *Hemophilus pertussis.* A lesser degree of sensitization was effected by gelatin, agar, dextran, and polyvinylpyrrolidone. Sensitization attained a maximum about ten days after the intraperitoneal inoculation of the sensitizing antigen, provided it was concurrent with or followed the adjuvant administration. The presence of circulating antibodies, titratable by the tanned-red-cell technique of Boyden at a time when anaphylactic sensitization is at its height, indicates the causal relationship. However, anaphylactic response can occur in the absence of titratable circulating antibody. None of the adjuvants was found capable of a simultaneous enhancement of histamine sensitivity.

The foregoing findings do not necessarily implicate the mode of action of *H. pertussis* as that of a classical "adjuvant."

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THE ROLE OF PLATELETS AND THE RELEASE OF SEROTONIN AND HISTAMINE DURING ANAPHYLAXIS IN THE RABBIT

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THE platelets of rabbit blood contain a relatively large amount of serotonin (5-hydroxytryptamine) and histamine as compared to other animal species[1-3] and man.[4-6] Rabbit whole blood and platelets have been very useful for studying the in vitro release of histamine.[7-10] Katz[10] first showed that the amount of histamine in the plasma was increased by incubating the blood from a sensitized rabbit with the corresponding antigen, indicating that histamine was liberated from the platelets by the antigen-antibody reaction. Numerous investigators have subsequently confirmed this finding.[7, 11, 12] Schachter[13] showed that histamine was also released in vivo in rabbits when the antigen was injected intravenously into previously sensitized animals. Humphrey and Jaques[14] incubated purified antigen and antibody with normal rabbit platelets suspended in plasma and found that the amount of both serotonin and histamine was increased in the plasma. Recently, Waalkes and colleagues[15] found that serotonin as well as histamine was liberated into plasma, both in vitro by the addition of antigen to the blood from sensitized rabbits and in vivo after the intravenous injection of the antigen into the rabbits.

During anaphylaxis the levels for whole blood serotonin and histamine were found to drop suddenly after injection of the antigen and to return to nearly normal values after several hours. The decrease in whole blood serotonin and histamine levels corresponded to the decrease in the number of circulating platelets in the peripheral blood. A similar drop in leukocytes occurred also during anaphylaxis. The fate of these blood elements was unknown. Since the number of platelets in the blood returned to normal levels after about two hours, however, it was thought that during anaphylaxis platelets, and probably leukocytes, might be trapped in various organs.[16] Because platelets contain large amounts of serotonin and histamine, an increase in the tissue content of these amines during anaphylaxis would suggest that platelets had been trapped. A prior study by Rose[17] did not reveal any difference in the histamine content of various rabbit organs during acute anaphylaxis as compared to the histamine content of the same organs in control animals.

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Received for publication Feb. 2, 1959.

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Because serotonin and histamine are liberated from platelets by in vitro antigen-antibody reactions, the release of these amines in vivo is assumed to be, in part, from platelets. The level of serotonin and histamine in the whole blood, but not in the tissues, of rabbits can be lowered by treating the animals with small amounts of reserpine. The results of anaphylaxis in rabbits treated in this manner suggest that histamine, but not serotonin, is liberated from tissues.[18]

The present investigation was designed to study the amount of serotonin and histamine in various organs during and after anaphylaxis in the rabbit. A rise in the serotonin and histamine content of any tissue would imply that platelets had lodged in that organ. In addition, after anaphylaxis a decrease in the amount of these amines below the normal range in any tissue would indicate their release from that tissue. Finally, the data obtained on serotonin in animals would be compared with available human data to study the possible role of serotonin in human hypersensitivity reactions.

MATERIALS AND METHODS

Male white rabbits, each weighing 1.5 kilograms, were sensitized to horse serum as previously described.[15] Siliconized glassware was employed wherever whole blood had to be handled. Blood for the in vitro studies was obtained by intracardiac puncture. Blood samples for the in vivo studies were obtained by means of a plastic tube inserted into the carotid artery. Anesthesia was induced by means of intravenous Nembutal supplemented with ether by inhalation. For the tissue studies, animals were killed at the stated time intervals by a blow on the head; the organs were removed immediately, cut into small pieces and pressed between pieces of porous paper to remove traces of blood, and finally homogenized in 0.1 N hydrochloride. Normal control animals were treated exactly the same as the experimental rabbits.

For precipitation of the insoluble antigen-antibody complex, a rabbit previously sensitized to horse serum was given a booster injection of 5 ml. of horse serum intraperitoneally. Blood was drawn after three weeks, and the serum was collected. The complex was found to precipitate immediately at a dilution of horse serum of 1:500 in n-saline with rabbit serum diluted 1:5 with n-saline. The precipitate was collected by centrifugation and washed three times with n-saline. The antigen-antibody complex was suspended in n-saline for the in vitro and in vivo studies.

In order to isolate platelets, 30 ml. of normal rabbit blood was drawn and the method of Dillard and associates[19] was used. The platelets were lysed by shaking with water and rapidly freezing. After thawing, sufficient hypertonic saline was added to make the final solution isotonic with blood.

Human lung was obtained from three patients by biopsy at the time of cardiac surgery. Blood from normal white mice was obtained, after induction of ether anesthesia, by opening the chest and drawing the blood from the heart.

Serotonin and histamine levels were determined in plasma, whole blood, and tissues by the method of Weissbach, Waalkes, and Udenfriend.[3]

TABLE I. SEROTONIN AND HISTAMINE CONTENT OF TISSUES DURING ANAPHYLAXIS*

ORGAN	SEROTONIN (γ/GM.)					HISTAMINE (γ/GM.)				
	NORMAL VALUES		AFTER HORSE SERUM			NORMAL VALUES		AFTER HORSE SERUM		
	AVERAGE	RANGE	TIME (MINUTES)	NON-SENSITIVE	SENSITIVE	AVERAGE	RANGE	TIME (MINUTES)	NON-SENSITIVE	SENSITIVE
Intestine	16.7	9.3-22.0	4	15.6	19.0	7.2	3.8-10.5	4	6.9	7.0
			15	13.0	13.7			15	6.6	6.8
			30	18.0	20.0			30	7.0	8.7
			60	13.2	13.0			60	5.9	6.2
			120	16.6	17.0			120	6.2	8.2
Liver	2.3	1.3-3.2	4	3.0	3.9	2.0	0.9-2.7	4	2.2	2.4
			15	1.8	6.6			15	1.8	2.5
			30	2.6	5.9			30	2.0	1.4
			60	2.8	2.9			60	1.5	2.2
			120	1.8	3.0			120	2.4	1.4
Brain	0.5	0.3-0.6	4	0.4	0.5	1.0	0.6-1.4	4	1.2	0.8
			15	0.5	0.5			15	1.0	0.9
			30	0.5	0.5			30	1.0	0.7
			60	0.5	0.4			60	0.8	0.9
			120	0.4	0.4			120	1.0	1.1
Heart	0.4	0.2-0.7	4	0.5	0.4	0.8	0.3-1.4	4	1.3	0.6
			15	0.4	0.3			15	0.9	0.8
			30	0.4	0.5			30	0.8	0.7
			60	0.5	0.4			60	0.8	0.8
			120	0.3	0.4			120	0.7	0.9

*Serotonin and histamine concentration was determined in tissues from the same animals used for the lung analyses in Fig. 1 and compared with normal values (average from fifteen rabbits) for same tissues from rabbits receiving no treatment.

RESULTS

Fig. 1 shows the lung levels for serotonin and histamine at specific time intervals during a two-hour period after the intravenous injection of the antigen into sensitized and normal control rabbits. The data show that both the serotonin and histamine concentrations in the lung of the sensitive animal rise rapidly after the injection of horse serum and gradually decrease following the peak values at four minutes. No significant change is noted in the lung values for the normal control animals.

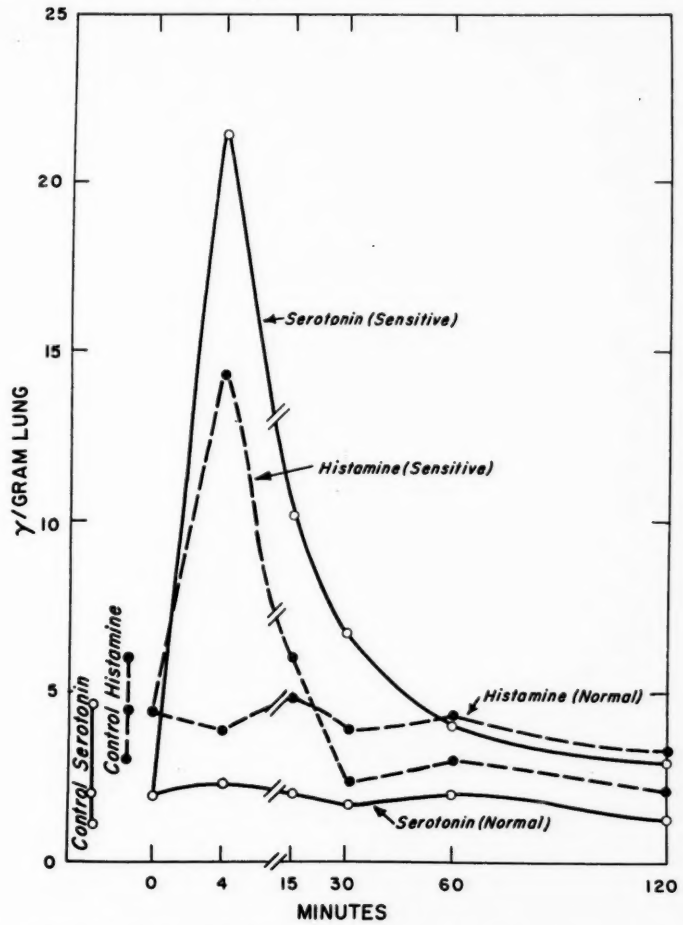

Fig. 1.—Lung serotonin and histamine during anaphylaxis. Horse serum (2 ml. per kilogram) was injected intravenously into both sensitized and normal control rabbits. Each point is the average value obtained from four animals killed at the corresponding time.

In Table I are shown the serotonin and histamine contents of other organs from the same animals represented in Fig. 1. Except for a slight increase in the average serotonin value for the liver of the sensitized animal, no distinct increase or decrease of serotonin or histamine is noted in any tissue during the two-hour period. The final two-hour values are also within the normal range for these amines.

Fig. 2 gives the whole blood values for serotonin and histamine over a two-hour period after the intravenous injection of dilute horse serum into sensitized and normal rabbits. Platelet and leukocyte counts on the same blood from the sensitive animals are also given.

Examination of the data for sensitized animals in Figs. 1 and 2 reveals that at the time of the peak concentration for serotonin and histamine in the lung, the whole blood and platelet levels are at their minimum values. The

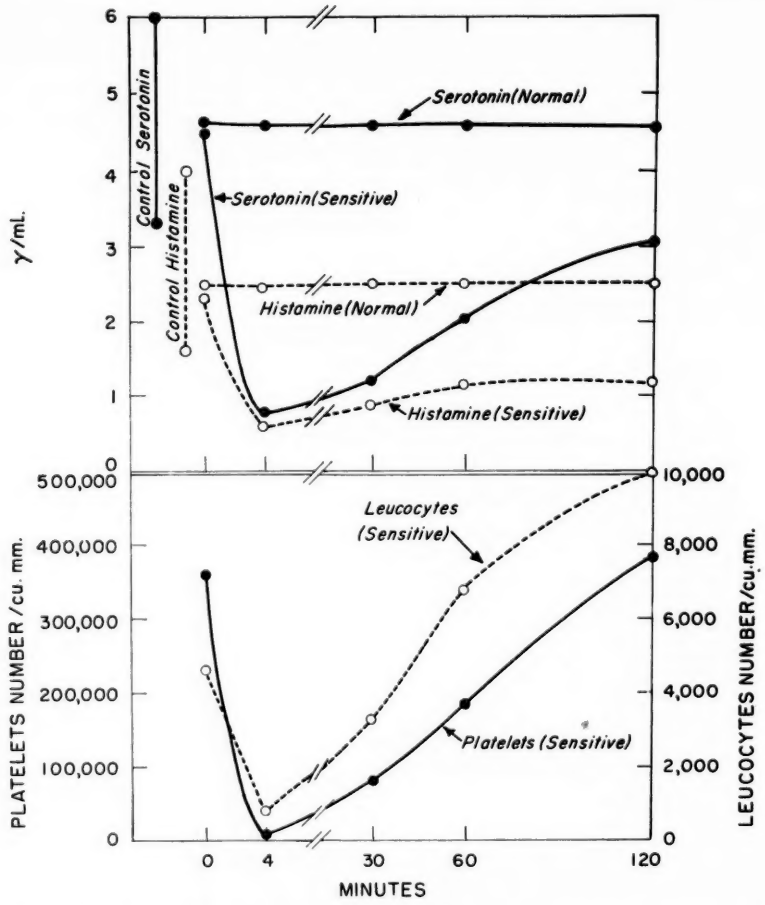

Fig. 2.—Whole blood serotonin, histamine, platelets, and leukocytes during anaphylaxis. Average whole blood serotonin and histamine, as well as leukocyte and platelet counts, were determined after the intravenous injection of horse serum (1:100 in saline; 1 ml. per kilogram) into two sensitized rabbits. Whole blood serotonin and histamine values were also obtained from normal control animals treated in the same manner.

decrease in platelets from the peripheral blood suggests that they have been either destroyed or trapped in various organs. The decrease in whole blood levels of serotonin and histamine, the rise in the lung concentration for these amines, and the return of the levels and of the number of circulating platelets toward normal values indicate temporary trapping of the platelets in the blood vessels of the lung during anaphylaxis.

Because serotonin and histamine are both liberated from platelets by antigen-antibody reactions in vivo, the release of these vasoactive amines could be a factor in the trapping of platelets and leukocytes in the lungs. The intravenous injection of serotonin (1 millimol of base per kilogram) and of histamine (1 millimol of base per kilogram), dissolved in the same n-saline solution, into normal rabbits caused death in four to five minutes. The major toxic symptom noted was marked respiratory distress. Tissues, including lung, intestine, brain, liver, and heart, were analyzed for serotonin and histamine but did not have an elevated level for these amines. No significant trapping of platelets in the lung or other tissues could have occurred as a result of the injection.

Platelets have been shown to be lysed during antigen-antibody reactions.[16] Products from the broken platelets might cause the formation of platelet and leukocyte clumps which are then caught in the lungs. The injection of lysed platelets into a normal rabbit, however, produced no change in whole blood serotonin and histamine and no increase in the amount of these amines in the lung or other organ.

TABLE II. SEROTONIN AND HISTAMINE CONTENT OF TISSUES DURING ANAPHYLAXIS AFTER INJECTION OF THE ANTIGEN INTO THE SYSTEMIC CIRCULATION (LUNG SEROTONIN AND HISTAMINE AFTER INJECTION OF ANTIGEN-ANTIBODY COMPLEX*)

	γ/GRAM					
	SEROTONIN			HISTAMINE		
TISSUE	EXPERIMENTAL	CONTROL	NORMAL RANGE	EXPERIMENTAL	CONTROL	NORMAL RANGE
Lung (A)	18.0	2.2	1.2-4.7	12.0	4.5	3.0-6.0
(B)	20.0			13.5		
Intestine	15.0	16.0	9.3-22.0	4.4	5.0	3.8-10.5
Liver	4.0	2.0	1.3-3.2	2.0	2.0	0.9-2.7
Brain	0.5	0.5	0.3-0.6	1.1	1.0	0.6-1.4
Heart	0.3	0.3	0.2-0.7	0.9	1.0	0.3-1.4

*Serotonin and histamine concentration in the lung (A) and other tissues during anaphylaxis was determined four minutes after the injection of the antigen (2 ml. per kilogram) into the systemic side of the circulation in sensitized rabbits. Serotonin and histamine in the lung (B) of normal rabbits were determined four minutes after the intravenous injection of the antigen-antibody complex (the amount precipitated from 13 ml. of rabbit serum used for each injection) suspended in saline. Each value is the average from two animals and is compared with the normal range.

The agglutination or clumping of platelets occurs immediately in vitro and in vivo when the blood from sensitized rabbits comes into contact with the antigen. In all previous work, the injection of the antigen has been by the intravenous route. The removal of the platelet emboli by the lungs may be due simply to the fact that the lungs are the first organ through which they pass. In order to determine whether other organs and tissues might act as a filter system for platelet-leukocyte clumps, the injection of antigen would have to be made into the left or systemic circulation. In Table II and Fig. 3 are presented the whole blood, plasma, and tissue values for serotonin and histamine during anaphylaxis produced by injection of horse serum into the systemic circulation by means of a catheter inserted in the left carotid artery and extended into the arch of the aorta. Probably only a small number of agglutinated platelets and leukocytes formed by injection of the antigen reach the brain or heart by this technique. Nevertheless, for the clumps to reach the lung, they would first pass

through the vascular beds of other organs and tissues. After the injection, the whole blood content for these amines decreased markedly, but the levels in the plasma were increased. The amount of serotonin and histamine in the lung at four minutes was elevated and to the same degree as that produced by the intravenous injection of the antigen into sensitized rabbits. The values for

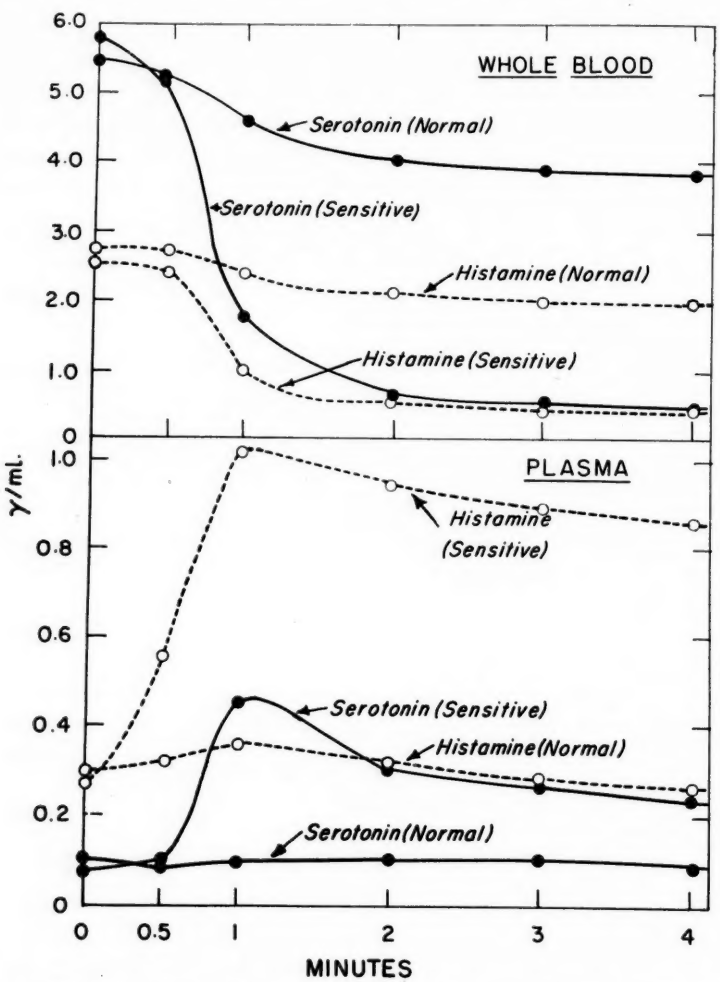

Fig. 3.—Whole blood and plasma serotonin and histamine during anaphylaxis after injection of the antigen into the systemic circulation. Whole blood and plasma serotonin and histamine were determined after injection of the antigen into the systemic circulation in those sensitized and normal control rabbits whose tissue values are given in Table II.

other organs were all within normal limits, except those for the liver which showed a slight increase in serotonin. Serotonin and histamine in whole blood, plasma, and tissues from control animals, treated in the same manner, were within the normal range except for a slight drop in the whole blood level for these amines. A similar decrease has been noted after the intravenous injection of 2 ml. per kilogram of horse serum into normal rabbits.

The results of the incubation of the antigen-antibody complex precipitate with normal rabbit blood and of horse serum with blood from rabbits sensitized to this antigen are listed in Table III. The antigen-antibody complex caused a

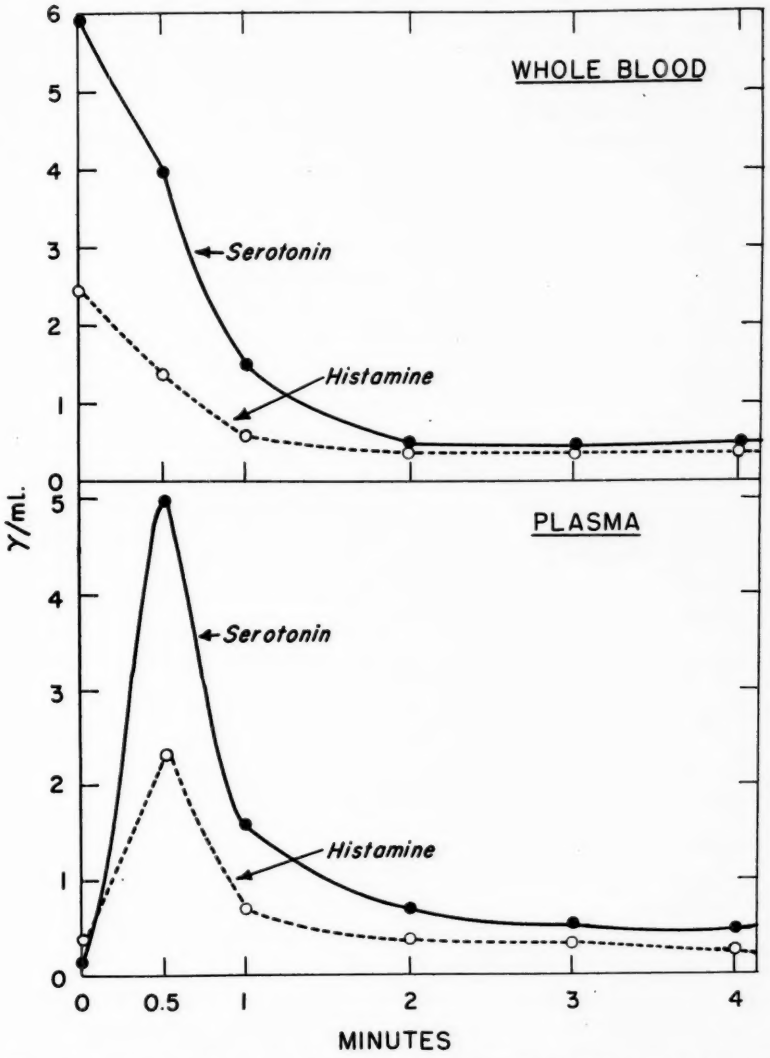

Fig. 4.—Serotonin and histamine in the blood of normal rabbits after injection of antigen-antibody complex. Rabbit whole blood and plasma serotonin and histamine concentrations were determined after the intravenous injection of the antigen-antibody complex precipitate into those normal animals whose lung value (*B*) is given in Table II.

release of serotonin and histamine from normal blood similar to the release of these amines when antigen is added to blood from a sensitized rabbit.

The intravenous injection of the antigen-antibody precipitate into a normal rabbit caused changes in the serotonin and histamine content of blood (Fig. 4) and lung (Table II) similar to the changes produced by the intravenous injection of antigen into sensitized rabbits. A marked drop in whole blood serotonin

and histamine concentration occurred, accompanied by an increased amount of these amines in the lungs, at four minutes. In addition, both serotonin and histamine were released into the plasma immediately after injection of the antigen-antibody complex.

TABLE III. RELEASE OF SEROTONIN AND HISTAMINE IN RABBIT BLOOD IN VITRO*

RABBITS	MATERIAL ADDED	NUMBER OF DETERMINATIONS	PLASMA (γ/ML.)	
			SEROTONIN	HISTAMINE
Sensitive to horse serum	1:100 horse serum	8	4.0	3.1
Sensitive to horse serum	n-Saline	8	0.4	0.5
Normal	1:100 horse serum	6	0.3	0.4
Normal	Antigen†-antibody complex	2	7.2	4.1
—	Antigen-antibody complex alone	2	0.0	0.0

*Whole blood was incubated at 37° C. for thirty minutes with 50 per cent by volume of saline solution (horse serum) or suspension (antigen-antibody precipitate). After centrifugation, the diluted plasma was analyzed for serotonin and histamine. The values were calculated on the basis of the original plasma.

†For each experiment, the amount of antigen-antibody precipitate used was obtained from 1.5 ml. of serum from sensitized rabbits.

In Table IV are compared the lung and blood concentrations of serotonin and histamine in rabbits, guinea pigs, mice, and human beings. Rabbits and mice have much more serotonin in the blood and lung than either guinea pigs or man. Histamine is much higher in rabbit blood than in the blood of the other species. Both guinea pigs and man have relatively large amounts of histamine in the lungs and essentially no serotonin.

TABLE IV. THE SEROTONIN AND HISTAMINE CONCENTRATION IN LUNG AND BLOOD OF VARIOUS ANIMAL SPECIES AND MAN

SPECIES	γ/ML. OR GM.			
	WHOLE BLOOD		LUNG	
	SEROTONIN	HISTAMINE	SEROTONIN	HISTAMINE
Rabbit	3.3-6.0	1.6-4.0	1.2-4.6	3.0-6.0
Mouse	2.1-4.6	<0.2	1.4-6.0	0.6-1.5
Guinea pig	<0.2*	0.11†	<0.2*	8.4-19.8
Human	0.09-0.18	0.11‡	<0.2*	4-20

*The symbol < indicates that a fluorescent reading but not a distinct curve was obtained for serotonin.

†Value taken from Code, C. F.: Am. J. Physiol. **127**: 78, 1939.

‡Value taken from Lowry and others: J. Pharmacol. & Exper. Therap. **112**: 116, 1954.

DISCUSSION

Anaphylaxis in the rabbit is accompanied by both a sudden release of serotonin and histamine into the plasma and a rapid, marked decrease in the whole blood content of these amines. The latter decrease is due to the drop in the number of circulating platelets. In addition to the loss of platelets in the peripheral blood, a simultaneous decrease in leukocytes occurs. The rise in the lung concentration of serotonin and histamine at the same time the amount of these amines in the peripheral blood is markedly reduced indicates that platelets (and probably the leukocytes) are trapped in the lungs during anaphylaxis. Analysis for these amines in other organs did not show any variation from the

normal range except in the liver, where a slight increase in serotonin was noted. When the antigen was injected into the systemic side of the circulation, the same results were obtained. The data indicate that the trapping of platelets during anaphylaxis is a property restricted largely to the lung.

Previous work has shown that antigen-antibody reaction in vivo in the rabbit causes clumping or agglutination of the platelets and leukocytes.[16, 20] The clumping is probably a major factor in the trapping of these blood elements in the lung. Clumping or agglutination also occurs in vitro when the antigen is added to whole blood from a rabbit sensitized to that antigen. Upon centrifugation for plasma of this blood after the incubation, the complete loss of the buffy coat is readily observed. The disappearance of the usual leukocyte-platelet layer after centrifugation of whole blood is always seen with the release of amines from the platelets not only in vitro but also in vivo during anaphylaxis. Because the release of serotonin and histamine from platelets also occurs in vitro, occlusion of the platelets in the lung is not necessary for the liberation of these amines.

The results of the in vitro and in vivo studies with the insoluble antigen-antibody complex in normal rabbits are the same as the in vitro and in vivo results in studies where sensitized animals and the specific antigen are used. These findings suggest that the results in the sensitized rabbits are mediated through the formation and action of this complex. The clumping of platelets and leukocytes and the release of serotonin and histamine from the platelets during anaphylaxis appear to be due to the direct action of the antigen-antibody complex itself and not a primary result of the interaction of antigen with antibody.

The in vivo decrease in platelets and leukocytes after injection of antigen into sensitized animals has been studied by numerous investigators. Webb,[21] and Dean and Webb,[22] in 1924, showed that leukocytes drop in the blood of dogs during anaphylactic shock. Code[23] reported an increase in whole blood histamine and a decrease in white blood cells during anaphylaxis in the dog. Leukopenia during anaphylaxis in the rabbit and the relationship with changes in blood histamine were discussed by Dragstedt and colleagues[24] in 1940. Kopeloff and Kopeloff,[25] working with sensitive monkeys and guinea pigs, reported a decrease in platelets in the blood after injection of the corresponding antigen.

The intravenous injection of other agents also produces this phenomenon of decrease in platelets and leukocytes in the blood. Rocha e Silva[16] reported that the injection of polysaccharides into normal rabbits and dogs would cause a drop in platelets and leukocytes. However, the platelets and leukocytes were thought to be distributed equally among all organs and not lodged in a specific tissue. Peptone[26] has been reported to cause shock and decrease in platelets and leukocytes in dogs. In 1938 Robertson and Yu[27] and more recently Weil and Spink[28] reported that the intravenous injection of endotoxin into dogs produced a drop in white blood cells and platelets analogous to anaphylactic shock. Intravenous trypsin in rabbits will produce a fall in platelets and leukocytes[29] and, in addition, a release of histamine and serotonin.[30] It is obvious that a variety

of agents, upon intravenous injection, are capable of producing a decrease in platelets and leukocytes. In each case the decrease is probably associated with clumping of these blood elements and distribution into one or more organs. Whether these various agents and the antigen-antibody complex all have or produce a specific factor in common in order to cause the disappearance of platelets and leukocytes from the peripheral circulation is a question of interest and importance.

In human beings, Storck and associates[31] have reported that the subcutaneous injection of ragweed antigen into patients sensitive to this antigen will cause a fall in the number of platelets in their blood. Whether platelets are caught in the lung or some other organ or tissue during allergic reactions is not known. However, we should consider the possibility that the formation of platelet-leukocyte clumps and the subsequent trapping of these clumps in a specific organ may occur and play a role in certain human diseases thought to be related to hypersensitivity states. Stetson,[32] for example, has found the occurrence of platelet-leukocyte thrombi, involving the smaller branches of the coronary blood vessels, in the hearts of patients with active rheumatic fever and rheumatic carditis. He observed a consistent correlation between the existence of these vascular lesions and the presence of typical Aschoff bodies and thought that these cellular thrombi might play a role in the pathogenesis of rheumatic carditis. Previous work[20, 33, 34] had shown that platelet-leukocyte clumps were involved in the formation of the Shwartzman and Arthus reactions in rabbits.

In human blood, serotonin is carried in the platelets[35] and histamine in the white blood cells.[4, 36] The amount of serotonin in human blood is much less than the amount in rabbit blood. The range for the serotonin content of human whole blood in normal adults is 0.09 γ to 0.18 γ per milliliter.[6] The total amount of serotonin in the blood of man appears to be too small to be of any great consequence during hypersensitivity reactions or anaphylaxis. However, if platelets are lodged in a specific tissue, as the lung, during anaphylactic shock and the serotonin is released, the amount of this amine which might be derived from blood alone could be a factor in the toxicity.

Examination of the tissue data in sensitized rabbits two hours after injection of the antigen shows that the levels for serotonin and histamine are all within normal limits. The lack of a decrease in tissue levels for these amines suggests that in the rabbit little serotonin or histamine is released from these organs during anaphylaxis. However, the intestine would be the principal source, particularly for serotonin. The normal range for these amines in the intestine is so great that only a massive release might be found by analysis. In evaluating the release of serotonin and histamine from tissues during anaphylaxis in the rabbit, caution must be observed, since the trapping of platelets could give a false impression of the true tissue level. After two hours, however, the platelet count should have returned to normal.

The release of histamine from guinea pig lung during antigen-antibody reaction in vitro and in vivo[37] is well recognized. The release of histamine from the lung in the living animal is considered to be of great importance during

anaphylactic shock. If human lung contained both serotonin and histamine, the release of these amines in man could be a vital factor during allergic reactions. Speculation as to the role of serotonin in the pulmonary aspects of hypersensitivity reactions has been made.[38] In some animal species in which both serotonin and histamine are present in the lung, it is difficult to attribute the toxicity seen during anaphylaxis to histamine alone, and both amines may contribute to the reaction. In the mouse, serotonin is present in the lung and also in the blood. Previous investigators have considered histamine to be of little importance during anaphylaxis in this species.[39, 40] Recently, Fink,[41] using the Schultz-Dale reaction, has shown evidence to implicate serotonin in mouse anaphylaxis. In the guinea pig, however, an animal whose lung contains essentially no serotonin and a relatively large amount of histamine, anaphylaxis could be explained on the basis of histamine release alone. Human lung has a relatively large amount of histamine and essentially no serotonin. Thus, the importance of the release of these amines from human lung during allergic reaction should be considered only as regards histamine.

Many studies on the mechanism of death during fatal anaphylaxis in the rabbit have been made.[42-44] Anaphylaxis is associated with a rise in the pressure in the pulmonary circulation and a fall in the pressure of the systemic circulation. Death has been attributed to acute right heart failure. Cohen and associates,[44] however, showed that the mechanism of death was also due, at least in part, to a peripheral respiratory failure. Herbertson[45] has shown a similar type of myocardial necrosis after anaphylaxis and after acute infarction of the lung. The rise in the pulmonary artery pressure is presumably due to constriction of the pulmonary vessels. Platelet and leukocyte emboli caught in the lung vessels could increase the occlusion of the blood supply, intensify the acute pressure changes in the pulmonary and systemic circulations, and increase respiratory distress. In addition, the large number of platelets caught up in the lungs permits the release of serotonin and histamine within the lung itself, which could produce an increased concentration of these vasoactive amines in the reactive lung parenchyma. Despite these severe changes which can occur, rabbits frequently do not die during anaphylaxis produced by horse serum.* This may be due to the fact that a considerable amount of the available serotonin and histamine is not released from the platelets or tissues. Several animals which died within two to four minutes after injection of the antigen had plasma and lung serotonin and histamine levels which were much higher than those for sensitized animals which had to be killed at this time.

SUMMARY

During anaphylaxis in the rabbit, whole blood serotonin and histamine decrease, plasma serotonin and histamine increase, and the lung content for these amines is transiently elevated. The drop in whole blood serotonin and histamine is due to the disappearance of the platelets which are caught in the lung. Injection of the antigen into the systemic circulation produced the same

*Fatal anaphylaxis, however, can be produced by the sensitization procedure used by Cohen and associates.[14]

results, indicating the phenomenon to be specific mainly for the lung. The in vitro results with normal blood and the in vivo effects in normal rabbits caused by the antigen-antibody complex were the same as those seen when a sensitized rabbit and the specific antigen were used. Speculation as to the role of platelet serotonin in human hypersensitivity reaction is made. The presence of histamine and the lack of serotonin in human lung as well as the relationship to human allergic pulmonary manifestations, are discussed. The pulmonary aspects of platelet thrombi in fatal anaphylaxis in rabbits are considered.

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THE EFFECT OF RESERPINE ON HISTAMINE AND SEROTONIN

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INTRODUCTION

RECENT investigations have shown that serotonin (5-hydroxytryptamine) as well as histamine must be considered as a possible factor in the hypersensitivity reactions in certain animal species.[1, 2] The role of serotonin in human allergy has not been clarified,[3] and the function of histamine is not fully understood. Further research in both animals and man is necessary before answers to these fundamental problems will be obtained. Studies of the release of histamine and serotonin by antigen-antibody reactions and also by chemical materials should yield important information. Many agents which liberate histamine from the cellular elements of blood and from tissues[4] have been found, whereas the release of serotonin by a variety of substances has not been studied as extensively.

Recently, attention has focused on reserpine, a drug which is useful in the treatment of several different diseases, including mental disorders and hypertension. The unique ability of reserpine to release specific vasoactive amines from a bound to a free form in blood and tissues has caused considerable speculation as to its mode of action. Previous studies by Carlsson and associates[5] showed that reserpine released serotonin in vitro from rabbit platelets. Haverback and co-workers[6] reported that reserpine administered orally to patients released serotonin from platelets, and Pletscher and associates[7] found that serotonin was liberated from animal tissues by this drug. In addition, Carlsson and Hillarp[8] showed that the intravenous injection of reserpine caused complete liberation of the catechol amines in the adrenal medulla of the rabbit, and Holzbauer and Vogt[9] reported a decrease in norepinephrine concentration of the hypothalamus of the cat after treatment with reserpine. Recently, Paasonen and Krayer[10] found that norepinephrine was released from both dog and rat heart by reserpine. In 1956, Waalkes and Weissbach[11] showed that intravenous reserpine liberated histamine in addition to serotonin from the platelets of rabbits. Although the release of these amines from platelets was similar, initial studies indicated that histamine in tissues was not affected to the same extent as serotonin.

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Received for publication Feb. 16, 1959.

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In all the investigations cited previously, the release of amines by reserpine was studied following a single injection of the drug. Usually reserpine is given to patients at intervals, and frequently for long periods of time. In order that any possible difference in the release of histamine and serotonin from animal tissues by reserpine might be determined, the present investigation was designed to study the concentration of these amines in tissues after a single injection and after multiple injections of the drug.

Platelets have been considered to bind amines and to function in part as carriers of specific amines.[12] Because of the relatively large amounts of serotonin and histamine present, rabbit blood and platelets are frequently used to study the release of these amines. The exact nature of the bond between platelets and either serotonin or histamine is not known. Whether the same type of linkage exists in other tissues is also unknown. Previous work had indicated a distinct difference in the ability of reserpine to release serotonin and histamine from normal rabbit platelets suspended in plasma.[13] To aid in a better understanding of the nature of the bonds between these active amines and both platelets and tissues, reserpine was incubated with rabbit whole blood and also with platelet-rich plasma in order to study the release of serotonin and histamine.

EXPERIMENTAL PROCEDURE

All glassware used to handle or hold blood and platelet-rich plasma was siliconized. Blood was obtained from rabbits by cardiac puncture. Heparin was used as anticoagulant when whole blood was incubated with reserpine. For platelet-rich plasma, 10 per cent by volume of 1 per cent disodium ethylenediaminetetraacetate in normal saline was used as anticoagulant, and the blood was treated in accord with the method of Dillard and associates.[14] Incubations were carried out in siliconized centrifuge tubes kept in a water bath at 37° C. Reserpine,* 20 γ per milliliter in 5 per cent dextrose in water, was added to the incubation tubes (6 ml. of whole blood or 4 ml. of platelet-rich plasma) to make a final dilution of 0.3 γ per milliliter as recommended by Carlsson and colleagues.[5] Control samples of the same blood or platelet-rich plasma, to which 5 per cent dextrose in water had been added, were run with each experimental tube. After the incubation and centrifugation, 2 ml. of plasma was used for determination of histamine and serotonin.

To study the effect on the histamine and the serotonin content of tissues after a single injection of reserpine, we injected 5 mg. per kilogram intravenously into male rabbits weighing 2 kilograms and intraperitoneally into guinea pigs and mice. The guinea pigs for this and subsequent experiments were all males of the same age, and each weighed 500 grams. The mice were of the same age, all males, and weighed 20 to 25 grams. After a single injection of reserpine, the guinea pigs and rabbits were sacrificed at twenty hours, and their tissues were homogenized in 0.1 N hydrochloride. The mice

*The reserpine for this study was donated by Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

were killed after twenty hours, and each whole mouse was homogenized in 0.1 N hydrochloride. Control animals were treated the same as the experimental ones and were given injections of 5 per cent dextrose in water.

To study the effect of multiple injections, we injected 2.5 mg. per kilogram of reserpine intraperitoneally in rabbits and guinea pigs every other day until a total of six injections had been given. Twenty hours after the last injection the animals were killed, and their tissues were homogenized in 0.1 N hydrochloride as before. Control animals were given injections of 5 per cent dextrose in water and maintained on exactly the same amount and kind of food as eaten by experimental animals. Since reserpine caused a loss of appetite and weight, the amount of the drug injected was decreased during the experiment in accord with any weight change. To study the effect of starvation on the histamine content of guinea pig lung, we gave six animals no food for seven days, after which they were killed and their lungs analyzed.

Histamine and serotonin in plasma, blood, and tissues were analyzed according to the method of Weissbach, Waalkes, and Udenfriend.[15] This method is of particular value in any comparative study involving these amines, since both histamine and serotonin can be determined on the same aliquot of a tissue homogenate or blood.

TABLE I. IN VITRO RELEASE OF SEROTONIN AND HISTAMINE*

SAMPLE	INCUBATION TIME (HOURS)	PLASMA (γ/ML.)			
		HISTAMINE		SEROTONIN	
		EXPERIMENTAL	CONTROL	EXPERIMENTAL	CONTROL
Whole blood	3.0	0.7	0.3	0.3	0.2
		1.3	0.4	0.3	0.2
	3.5	1.3	0.4	0.3	0.2
		2.3	0.4	0.3	0.2
	4.0	0.6	0.3	0.3	0.3
	4.5	0.6	0.2	0.2	0.2
		0.5	0.2	0.2	0.3
	5.0	0.9	0.3	0.1	0.3
		1.0	0.4	0.2	0.4
Platelet-rich plasma	5.0	1.3	0.3	3.5	0.1
		1.6	0.5	4.0	0.2
		1.4	0.4	4.1	0.3
		1.6	0.4	4.5	0.3
		1.1	0.4	2.9	0.2
		1.0	0.4	3.0	0.3

*Whole blood or platelet-rich plasma was incubated with reserpine in 5 per cent dextrose in water so that the final concentration of the drug was 0.3 γ per milliliter. Plasma histamine and serotonin were determined after centrifugation.

RESULTS

Table I shows the effect of reserpine on the platelet content of histamine and serotonin after incubation with whole blood or platelet-rich plasma. Histamine was released into the plasma in both experiments. Incubation for longer than five to six hours was found to cause platelet rupture and elevated control plasma levels for these amines. Serotonin was liberated by reserpine from platelet-rich plasma as shown by Carlsson and associates.[5] When whole

blood was incubated with reserpine, however, the subsequent serotonin values were low and essentially equal to those of the control samples. Further investigation has shown that free serotonin is metabolized to 5-hydroxyindoleacetic acid by rabbit blood but not by platelets or plasma alone.[16]

Table II gives the values for histamine and serotonin in guinea pig and rabbit tissues and in whole mice. The data show that serotonin is liberated readily from the tissues of these animals after a single injection of reserpine. Both histamine and serotonin are released from rabbit platelets to a marked degree after one injection of reserpine. The liberation of these amines in vivo is shown by the drop in their whole blood values. The histamine content of guinea pig and rabbit lung decreases significantly after one injection of reserpine, and a further decrease is noted after multiple injections of the drug. No significant change in histamine content of rabbit and guinea pig brain or of whole mice was noted after a single injection of reserpine. Little change was found in the histamine content of guinea pig intestine after a single injection or after multiple injections of reserpine. A significant decrease was noted in the histamine concentration of rabbit intestine only after multiple injections of the drug.

Reserpine, in the dosage used for the repeated injections, was found to be toxic for guinea pigs. Decline in appetite and weight loss, more than would be expected by starvation alone, were noted. To determine the effect of starvation on the histamine content of guinea pig lung, we gave six animals no food for a period of seven days. Analyses of their lungs after that period gave results similar to the control values for histamine, as shown in Table II, with an average concentration of 15.7 γ per gram.

DISCUSSION

Reserpine, after a single intravenous injection, has been shown to cause a release of histamine and serotonin from rabbit platelets.[11] The reduction in histamine was similar in rate and degree to the decrease in serotonin. The return to normal levels for both of these amines was also similar. Recent evidence has shown that agents such as trypsin, polysaccharides, and antigen-antibody complex, which in vitro and in vivo rapidly release histamine from rabbit platelets, also liberate serotonin in a similar manner.[17] In addition, compound 48/80 and other chemicals which do not release histamine from rabbit platelets do not release serotonin. Thus, so far studies have shown that agents which liberated histamine from rabbit platelets also freed serotonin; agents which did not liberate histamine did not release serotonin.

Although serotonin is readily liberated from brain, lung, and intestine, histamine in these tissues appears to be much more resistant to the action of reserpine. Incomplete studies in the rat and dog indicate similar results.[17] A single injection of reserpine into mice did not change the total histamine content as compared to the total amount in control animals. The serotonin content, on the other hand, decreased, indicating a definite release of this amine. In mice the skin contains much more histamine than other tissues

TABLE II. IN VIVO EFFECT OF RESERPINE ON TISSUE HISTAMINE AND SEROTONIN*

ANIMAL	ORGAN	TREATMENT	NUMBER OF ANIMALS	HISTAMINE γ/GM. OR ML. (AVERAGE)	HISTAMINE RANGE	HISTAMINE S.E.	HISTAMINE P	SEROTONIN γ/GM. OR ML. (AVERAGE)	SEROTONIN RANGE
Guinea pig	Lung	Control	16	15.1	8.4-19.8	0.904	—	<0.25*	—
		Reserpine							
		1 injection†	13	11.1	6.2-15.2	0.843	<0.001		
		6 injections	9	6.2	4.3- 7.4	0.343	<0.001		
		No food 1 week	8	15.7	8.9-20.0	—	—		
	Intestine (sm.)	Control	10	13.9	10.5-17.5	0.736	—	7.1	6.5-7.7
		Reserpine							
		1 injection	13	13.8	9.4-17.2	0.699	>0.45	0.6	0.5-0.8
		6 injections	9	11.8	10.2-13.3	0.353	0.013	0.7	0.6-0.8
	Brain	Control	10	1.0	0.8- 1.2	—	—	0.4	0.4-0.5
		Reserpine	13						
		1 injection		1.0	0.8- 1.2	—	—	<0.1	<0.07-<0.13
Rabbit	Whole blood	Control	20	2.7	1.6- 4.0	—	—	4.6	3.3-6.0
		Reserpine							
		1 injection	10	0.7	0.5- 1.0	—	—	0.6	0.4-0.9
	Lung	Control	12	4.4	2.7- 5.9	0.304	—	2.0	1.2-4.6
		Reserpine							
		1 injection	6	3.0	2.3- 3.2	0.160	<0.001	0.3	0.2-0.5
		6 injections	4	1.6	1.2- 1.9	0.177	<0.001	0.3	0.2-0.4
	Intestine (sm.)	Control	12	7.2	4.0-10.5	0.613	—	16.7	9.3-22.0
		Reserpine							
		1 injection	6	5.5	5.0- 6.0	0.165	0.03	4.0	2.7-5.2
		6 injections	4	2.5	2.3- 3.0	0.161	<0.001	1.8	0.9-2.5
	Brain	Control	12	1.0	0.6- 1.4	—	—	0.5	0.3-0.6
		Reserpine							
		1 injection	6	0.6	0.5- 0.7	—	—	<0.2	<0.1-<0.2
Mouse	Whole animal	Control	10	7.7	6.5- 9.1	—	—	1.8	1.6-2.0
		Reserpine 1 injection	10	7.1	6.0- 8.3	—	—	0.8	0.7-0.9

*The amount of histamine and serotonin in tissues was determined after reserpine treatment.

†For one injection, 5 mg. per kilogram was given; for multiple injections, 2.5 mg. per kilogram was administered every other day. The animals were killed twenty hours after the final injection.

and is essentially devoid of serotonin. Studies have also been conducted with mice harboring a transplantable, subcutaneous mastocytoma[18] containing large amounts of histamine and serotonin.[19] After a single intraperitoneal injection of reserpine into these mice, histamine in the tumors decreased significantly as compared to the tumors growing in the control animals. The concentration of serotonin in the experimental tumors was lowered to a much greater extent, however, than the histamine concentration.[20]

The evidence suggests that serotonin and histamine are held in platelets by a similar type of linkage. In tissues, however, serotonin and histamine may be bound in a different way. This is further suggested by evidence that antigen-antibody reactions in rabbits appear to release histamine but not serotonin from tissues.[21]

Whether reserpine interferes with the synthesis of histamine in the living animal is not known. Large doses of reserpine have been thought to produce changes in normal metabolic patterns.[22]

Reserpine frequently causes nasal stuffiness in patients after prolonged use. Antihistaminics are of some value in treating this as well as other toxic side effects sometimes noted with reserpine therapy. An increase in the volume and free acidity of gastric secretion is found in patients after oral or parenteral administration of reserpine.[23] It has been considered that the stimulus of reserpine to cause these changes in gastric secretion was mediated by serotonin. Recent data by Haverback and co-workers[24] show that serotonin actually decreases gastric secretion of acid in the Heidenhain pouch and that 5-hydroxytryptophan, a precursor of serotonin, inhibits spontaneous secretion but does not block the gastric response to reserpine or histamine. In view of these findings, as well as the data presented here, there is evidence to suggest that the action of reserpine in causing increased gastric secretion and nasal stuffiness may be mediated through histamine release. As a point of comparison, human lung, similar to the guinea pig, has a relatively large amount of histamine.[17] Whether the release of histamine is of any importance in the therapeutic aspects of reserpine is speculative.

SUMMARY

1. Reserpine liberates serotonin readily from blood and tissues and liberates histamine from rabbit blood. In guinea pigs and rabbits histamine was found to be released in part from tissues by reserpine but not as readily or to the same extent as serotonin.

2. The evidence suggests a possible similar type of bond for both histamine and serotonin in platelets but a different type of linkage in tissues.

3. The role of reserpine in producing side effects in patients by histamine release is suggested and discussed.

The authors wish to acknowledge the kind assistance of Mrs. Lila K. Randolph, Cancer Chemotherapy National Service Center, in the preparation and analysis of the data.

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ANTIANAPHYLACTIC AND ANTISEROTONIN ACTIVITY OF A COMPOUND OBTAINED FROM EGG YOLK, PEANUT OIL, AND SOYBEAN LECITHIN

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With the Technical Assistance of John Bladzinski

INTRODUCTION

SEVERAL investigators have indicated that extracts of egg yolk, peanut oil, and soybean lecithin have anti-inflammatory activity.[1-3] Purification of these anti-inflammatory extracts was achieved, and in each case the active agent was identified as N(2-hydroxyethyl) palmitamide.[4] This compound was assayed in the guinea pig by measuring reduction of swelling induced in joints by an antigen-antibody reaction. Swelling was reduced about 60 per cent at low concentrations (3 γ per kilogram), and tuberculin reaction in the guinea pig was inhibited.[3, 5] On the other hand, no activity was found by several other types of assays employed to measure anti-inflammatory activity.[3] The findings in the guinea pig prompted us to investigate the effect of ethanolamine palmitamide and related compounds on systemic anaphylaxis and on serotonin and histamine toxicity in the mouse.

MATERIALS AND METHODS

Female mice (Swiss Webster mice from Tumblebrook Farms, Bryant Lake, New York), each weighing from 14 to 16 grams, were used. The mice were fed Purina Laboratory Pellets and had free access to water. All drugs used in this study were diluted in a phophate buffer, pH 7.2.

Passive Anaphylaxis.—Mice were injected intraperitoneally with 2 billion killed smooth *Bordetella pertussis* cells suspended in 0.25 ml. saline. Four days later they received intraperitoneal injections of 0.15 to 0.2 ml. of a rabbit anti-egg albumin serum (250 γ antibody nitrogen). A half-hour later the drug to be tested was injected intraperitoneally, and one hour thereafter the mice were challenged intravenously with 0.5 mg. of commercial crystalline egg albumin in 0.2 ml. of saline. The number of survivors was recorded after sixteen to twenty hours.

Histamine Sensitivity.—Mice were sensitized to histamine by intraperitoneal injection of 2 billion *Bordetella pertussis* cells as described by Munoz.[6] Four days later they were challenged by intraperitoneal injection of 0.5 mg. of histamine base. All drugs tested were given one hour before challenge. The number of surviving animals was recorded twenty hours later.

From the Merck Institute for Therapeutic Research.
Received for publication Feb. 2, 1959.

Serotonin Sensitivity.—Using the procedures described by Munoz,[7] we sensitized mice with *B. pertussis* and four days later challenged them intravenously with 10 mg. per kilogram of serotonin base. All treatments were given intraperitoneally one hour before challenge. The number of survivors was recorded after twenty hours.

RESULTS

Anaphylactic Shock.—The data presented in Table I indicate that ethanolamine palmitamide protects mice against fatal anaphylactic shock when given in a dose of 0.5 to 5 mg. per kilogram. Both ethanolamine hydrochloride and

TABLE I. EFFECT OF ETHANOLAMINE PALMITAMIDE, ETHANOLAMINE HCl AND FATTY ACIDS ON PASSIVE ANAPHYLAXIS IN THE MOUSE

DRUG	DOSE (MG./KG.)	SURVIVAL/TOTAL	PER CENT SURVIVAL
N(2-hydroxyethyl) palmitamide	50	21/30	70
	25	5/8	62
	5	18/30	57
	0.5	19/30	63*
	0.05	10/20	50
	0.005	6/20	30
Ethanolamine HCl	50	18/29	62
	25	9/20	45
	5	20/29	69*
	0.5	13/30	43
	0.05	10/20	50
	0.005	7/20	35
Palmitic acid	50	19/30	63*
	5	14/30	47
	0.5	4/15	27
	0.05	4/15	27
	0.005	3/10	30
Hydrocortisone	100	19/25	76
Control	—	15/60	25

*The lowest active dose P = <0.001.

palmitic acid were also found to be effective in preventing death in this kind of experiment. When activity was obtained with any of the foregoing preparations, higher dosage did not increase the number of surviving animals. This appears, therefore, to be an "all-or-none" type of reaction. These experiments were repeated several times, and similar results were obtained. However, as the degree of sensitization increased and mortality of controls reached close to 100 per cent, higher doses were needed and fewer animals were saved by this drug. In this respect, it behaved similarly to hydrocortisone.

Antiserotonin Activity.—It has been known for some time that both histamine and serotonin are involved in anaphylaxis in the mouse. We felt, therefore, that it would be of interest to determine the effect of N(2-hydroxyethyl) palmitamide on serotonin and/or histamine sensitivity in the mouse. The data presented in Table II indicate that N(2-hydroxyethyl) palmitamide, 0.5 mg per kilogram, is highly effective in preventing death from serotonin

toxicity in the mouse. In order to protect the mice with ethanolamine hydrochloride, 500 times this amount was needed (250 mg. per kilograms). On the other hand, the palmitic acid moiety was found to be active at 0.5 mg. per kilogram, and the question of whether this was a specific property for palmitic

TABLE II. EFFECT OF N(2-HYDROXYETHYL) PALMITAMIDE AND ITS COMPONENTS ON SEROTONIN TOXICITY IN THE MOUSE

DRUG	DOSE (MG./KG.)	SURVIVAL/TOTAL	PER CENT SURVIVAL
N(2-hydroxyethyl) palmitamide	50	72/85	84
	5	41/70	58
	0.5	15/35	42*†
	0.05	6/35	17
	0.005	1/14	7
	0.0005	2/10	20
Palmitic acid	5	16/25	64
	0.5	8/15	53*‡
	0.05	5/10	50
	0.005	3/10	30
Ethanolamine HCl	250	16/30	53*§
	150	5/15	33
	100	4/10	40
	50	7/25	28
	5	3/15	20
Control	—	17/75	22

*The lowest active dose.
†P = 0.05.
‡P = <0.05.
§P = <0.01.

acid or a nonspecific one shared by other fatty acids and completely unrelated compounds was raised. Data presented in Table III indicate that a great variety of fatty acids are capable of protecting mice against serotonin toxicity. To test the specificity of this inhibitory reaction, we assayed a variety of other drugs for activity. At equivalent dose levels, ethanolamine hydrochloride and sarcosine were active whereas 1-amino-2-propanol was inactive. Similarly, urethane and pentobarbital sodium at 5 mg. per kilogram were inactive.

TABLE III. EFFECT OF FATTY ACIDS ON SEROTONIN TOXICITY IN THE MOUSE

COMPOUND	DOSE (MG./KG.)	SURVIVAL/TOTAL	PER CENT SURVIVAL
Control	–	20/100	20
Palmitic acid	5	41/70	58
Lauric acid	5	35/55	63
Caproic acid	5	16/30	53
Butyric acid	5	20/30	67
Na acetate	5	10/30	33*
Oleic acid	5	27/40	68
Linoleic acid	5	27/50	54
Ethyl linolenate	5	24/40	60

*Not active, all others P = <0.001.

Antihistamine Effect.—The compounds that proved effective against serotonin toxicity were also tested for protective effect against histamine sensitivity and were found to be inactive at either 5 or 50 mg. per kilogram per dose.

DISCUSSION

All previous work with crude extracts of egg yolk, soybean lecithin, and peanut oil, and later with ethanolamine palmitamide, was performed in the guinea pig.[1-3] In this species, the foregoing materials were highly active in reducing swelling induced by antigen-antibody reaction and also in reducing tuberculin reaction. Since the guinea pig's physiologic behavior is frequently different from that of other species of animals, and since the methods of testing activity in the guinea pig were somewhat complicated, a search was made in other species for other biologic activities of this compound. Results presented here indicate that these compounds also are highly active in preventing passive anaphylaxis in the mouse. The number of survivors seen after treatment with 0.5 mg. per kilogram of palmitamide was about equal to that seen with 100 mg. per kilogram of hydrocortisone.

Furthermore, it has been shown that N(2-hydroxyethyl) palmitamide and variety of fatty acids can inhibit serotonin toxicity in the mouse without affecting histamine toxicity. This activity was found not only in fatty acids of high molecular weight but also in butyric acid with only four carbons. Similarly, the degree of saturation of these fatty acids did not seem to be important, since oleic acid, linoleic acid, and ethyl linolenate were equally active. It would be hard to believe that a fatty acid would act as a specific antiserotonin compound, and it must therefore be assumed that its pharmacologic action is nonspecific.

Preliminary experiments in cats have also shown that palmitic acid given intraperitoneally in doses of 25 mg. per kilogram inhibits blood pressure changes induced by 3 to 5 γ per kilogram of serotonin while the responses to either histamine or epinephrine are not affected. Serotonin's mode of action in the cat is not very well understood at present, but it is believed to be due to a coronary chemoreflex and an inhibition of neurogenic vasoconstriction resulting in a depressor effect.[8] As the mechanism of action of serotonin is not known, one can only speculate on the mode of action of the inhibitory effect of palmitic acid and hope that future studies will throw some light on this problem.

In recent years, the injection of *B. pertussis* vaccine into mice has been shown to make the mouse more sensitive to the action of histamine, serotonin, anaphylaxis, cold stress, and a variety of other forms of stress.[9] These results suggest that some basic physiologic change is induced by *B. pertussis,* rather than a change which sensitizes the animal to particular noxious agents, such as histamine. Since serotonin but not histamine toxicity was affected, it must be assumed that these fundamental physiologic alterations brought about by *B. pertussis* in the mouse are not changed by either ethanolamine palmitamide or the fatty acids. At slightly higher dosage of the drug, adrenalectomized mice can also be protected against serotonin; this indicates that its action is independent of the adrenals. To substantiate this fact, it has been shown that this compound does not influence adrenal weight, corticosterone content of

adrenals, or thymus weight.[10] The action of this compound cannot be attributed to anticomplementary activity or to changes in the clotting mechanism, as prothrombin time was unaffected at physiologic concentrations.[11]

Little if any attention has been paid to the physiologic and pharmacologic action of fatty acids. The finding, therefore, that fatty acids could act as potent antiserotonin and antianaphylactic agents in the mouse was unexpected and opens a new area for study. No data are available on the amount of free fatty acids in the blood. It would be hard to conceive that a small amount of a particular fatty acid, given either orally or intraperitoneally in these experiments, is far in excess of that present in the normal diet. Woolley[12] believes serotonin acts by transporting calcium ions across a lipoidlike cell membrane, suggesting that a fatty acid, possibly preferentially adsorbed or solubilized, could block this action.

SUMMARY

The effect of N(2-hydroxyethyl) palmitamide, ethanolamine hydrochloride, and a variety of fatty acids on passive anaphylaxis, serotonin, and histamine toxicity in the mouse was studied. The above compounds were found to be active in suppressing anaphylaxis and serotonin toxicity at low concentrations (about 5 mg. per kilogram), but they were shown to have no effect on histamine toxicity. Data presented indicate that fatty acids can act as potent pharmacologic agents.

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THE PROBLEM OF NASAL POLYPS

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ONE of the unsolved and vexing problems in rhinology is the prevalence of polypoid changes in the nasal mucosa, manifesting themselves as single or multiple bodies known as polyps. The vexation, which in reality is frustration on the part of the clinician, is the polyps' variability of response to therapy, particularly their tendency to recur in spite of measures which some observers have reported to be adequate.

Many disciplines, particularly those of the rhinologist and the allergist, have been concerned over this local manifestation. The uncertain etiology of nasal polyps has led to a fragmentation of effort which has produced an unhealthy and paradoxical state of affairs. The rhinologist has essayed the role of the allergist, and the latter has assumed the responsibilities of the former. Perhaps this has arisen as the result of the dissatisfaction that each group has experienced at the hands of the other. Unfortunately, the plight of the middle man, the patient, is not improved by this dichotomy.

One would be abashed if he were to study the historial documentation of the past and compare it with today's so-called "modern concepts." Even in the fifth century B.C., Herodotus stated: "The art of medicine is thus divided among them: Medicine is practiced among them on a plan of separation; each physician treats a single disease and no more; thus the country swarms with medical practitioners, some undertaking to cure diseases of the eye, others of the head, others again of the teeth, others of the intestines, and some those which are not local."

Montaigne not only credited Herodotus but also approved of his views, for he said: "The Egyptians were right in neglecting the general calling of physician and of dividing the profession; for each illness, for each part of the body, there was an attendant, and therefore each part was more skillfully and less blindly treated, because they studied each one specially."

Nasal polyps have been prominently discussed in medical history from the earliest times. Pliny is frequently quoted as saying that for 600 years Rome was without physicians but not without physic. We know that Cato the Censor (born in 232 B.C.) had a very poor opinion of doctors and, in fact, of learning in general. Amid many recipes, amulets, charms, and invocations, we find him prescribing his favorite and almost his only drug for nasal polyps:

From the Department of Otolaryngology, University of Illinois College of Medicine.

Presented before the American Academy of Allergy, fifteenth annual meeting, Chicago, Illinois, Feb. 11, 1959.

Received for publication Feb. 10, 1959.

"If there is a polypus in the nose, rub together some dry wild cabbage leaves in the hand and place it at the nose and draw up the breath as much as you can. In three days the polypus will fall away. Nevertheless, for some days do the same; so that you may render the roots of the polypi entirely healthy."

A thousand years before Christ, one of the methods recommended for causing sneezing involved looking at the sun so that its rays would fall on the mucous membrane of the nostrils. There was mentioned an instrument, supposedly some sort of curette, for "eradicating nasal polypi; a frequent and troublesome disease in many parts of Hindustan." The Hindu idea was that all bodily diseases come from Wind, Bile, and Phlegm. Polyps were believed to be caused by Phlegm.

The world's first rhinologist, Hippocrates (460-370 B.C.), is credited with developing a technique for removal of polyps. After a sponge of sufficient size to fill the nasal cavity had been selected, four strings, each 1 cubit in length, were attached to it and their free ends were tied together. A long, flexible, metal probe with an eye at one end was next passed through the nostril and brought out at the mouth; the united ends of the strings were threaded through the eye of the probe, which was then drawn through the nose. The strings were now seized by the operator and, by forcible traction, the sponge was drawn through the nose, the mass of the polyp coming with it. Regardless of whether the growths were removed by evulsion or by cautery, Hippocrates afterward applied a dressing consisting of honey, to which was occasionally added some strong caustic; this was kept in contact with the parts by means of small leaden plates inserted into the nostrils. In the case of hard polyps, Hippocrates directed that the nostril should be slit open in order that the tumor might be thoroughly extirpated and the roots afterward destroyed with a hot iron through a hollow tube used as a speculum.

In another method for removing harder growths, perhaps what we identify as fibrous hypertrophies, Hippocrates employed the principle of the snare. The loop of a sinew was adjusted around the polyp and, after the end had been carried to the pharynx and traction had been made as before, evulsion was attained.

Aurelius Cornelius Celsus, who probably was born at the beginning of the Christian era and who lived during the reign of Tiberius Caesar, likened the appearance of polyps to the nipples of a female breast. He described them as showing in front on the lip and sometimes behind, "by that foramen through which the breath descends to the fauces, hanging down so that it may be seen behind the uvula, and in cold, damp days it strangles a man." He treated some polyps by caustics. To remove others, he used a sharp spatula-like instrument to separate the polyp from bone and a hook to remove the polyp. He strongly disapproved of meddling with the harder tumors, which he considered malignant. It was Claudius Galen (A.D. 131-201), however, who divided diseases of the nose into two classes, polyps and ozena. While his differentitation of disease was somewhat faulty, he did state: "Sarcoma is the unnatural growth of flesh within the nostrils. Indeed, a polyp is a kind of sarcoma. . . . A sarcoma differs from a polyp in size and structure."

The Arabians, through the knowledge gained from Paulus of Aegina (A.D. 625-690), followed the technique of Hippocrates:

> Taking, then, a thread moderately thick, like a cord, and having tied knots upon it at the distance of two or three fingers' breadth, we introduce it into the opening of a double-headed specillum [probe] and we push the other extremity of the specillum upward to the ethmoid openings, passing it by the palate and mouth, and then drawing it by both hands, we saw away, as it were, with the knots the fleshy bodies. After the operation, we keep the opening separate by means of a tent resembling the wick of a lamp.

One might add the remarks of Paulus of Aegina to the technique:

> After the operation, having sponged the parts carefully, we inject oxycrate of wine into the nose, and if the fluid descend by the roof of the mouth into the pharynx, the operation will have been rightly done; but if not it is clear that about the ethmoid bones, or upper part of the nose, there are fleshy bodies which have not been reached with the polypus instruments.

Paulus of Aegina was the first to identify the ethmoid cells with the origin of nasal polyps. However, the pathogenetic merry-go-round began with Ibn Sina, or Avicenna (980-1037), known as the "Prince of Physicians," who wrote the *Canon of Medicine*. He likened polyps to hemorrhoids and advised the use of a ligature for their removal.

The ablest Italian surgeon of the thirteenth century, Guglielmo Salicetti, called Saliceto or Salicet (about 1210-1277), proposed the gradual dilatation of the anterior openings of the nostrils with a sponge or some other device to facilitate the avulsion of nasal polyps. The knotted strings of horsehair and of silk were soon abandoned after the Renaissance. In 1571 Aranzio, or Arantius, in giving an account of his method of dealing with polyps, described not only a forceps with long jaws which he had invented for the purpose but also a method that he had developed for illuminating the nasal cavity by darkening the room completely except for a ray of light which would be permitted through a hole made through a wooden shutter. On a cloudy day he used artificial light magnified by a water bottle.

Another Arabian, Johannes Mesua Damascenus, advised the use of forceps for the removal of polyps, followed by cauterization of their roots, or else the use of hot forceps. If this method was impossible, he used horsehair string. About this same era, Abulkasim, called Albucasis, reported the following case:

> Once I treated the tumor of a woman which had grown into her throat. It was of a dusky color and not very sensitive. The woman was almost strangled, and from the constriction of the passage breathed with difficulty and she was prevented from drinking or eating anything, so that she was reduced almost to the point of death, and she was in this condition a day or two. The tumor so projected forward that two branches of it protruded from the nostrils. Therefore with the greatest promptitude I hastened to fix in one of these a hook and dragged on it, and that whole portion was pulled forward. Then I cut it off where I had pulled it out at the nostrils. Then I did the same for that which projected on the other side. Then I opened her mouth and depressed her tongue. Then I fixed a hook in the tumor itself and cut off a part of it and only a little blood came from it, and the throat of this woman was free and she immediately drank water. Then I offered her some food. I did not cease to cut pieces from this tumor for a long time but the new growth filled the place of the excised pieces until her patience and my

own were exhausted. Wisely then did I act, and I cauterized the tumor up in the back of the throat and thereupon it did not recur. Then the woman left me and I know not what God did with her after me.

How like today! Yet, even Albucasis was indeed backward in utilizing techniques that Hippocrates had employed 1,500 years before him.

While amulets and charms disappeared from medicine soon after the beginning of the Renaissance, astrology long continued popular. Not only were stars consulted but physicians gave discourses on the influence of the moon on nasal polyps. Among men of science, however, astrology soon became astronomy, alchemy became chemistry, and therapeutics began to look to physiology and pathology for help.

In 1591, Forestus noted, in a discussion of anosmia: "If it is from ethmoidal obstruction, or from the humor discharged from a catarrh, the latter must first be cured. If from the flesh growing from within the nose, or from a wart or a hemorrhoid, it is to be cured by the surgeons by operative procedures, either with a cutting instrument, or cautery, or snare." Forestus had noted the case of a woman in whose nostrils a large polyp had grown "due to carrying heavy weights on her head; it forced the mucus down into the membranes of the nose."

Gabriele Fallopio, or Fallopius (1523-1562), went further with the Arabian hemorrhoidal theory in describing two types of growth—(1) vascular hypertrophies, which were to be found farther back in the nose, and (2) polyps, which were edematous growths situated in more accessible parts of the nose. For the cure of the anterior growths, he used a ligature, leaving it around the growth for two or three days, when it would fall off with the constricted mass. He stated:

> But when the polypus is well within the nose it is difficult to use the ligature, which should encircle the roots of the polyp. I take a silver tube which is neither too broad or too narrow, and then a brass or steel wire, sufficiently thick, preferably the iron wire from which harpsichords are made. This double I place in the tube so that from this wire loop is made one end of the tube, by which, used in the nares, I remove the polyp. When the polyp is engaged in the loop, I push the tube to the root of the polyp and extract it, since by this wire loop the root of the polyp is cut because it is a soft substance.

Fallopius pulled harder growths down, so that he could cut off the roots with a knife.

In 1628 Glandorp published a treatise on polyps. This work was remarkable for its erudition; moreover, it contained a very accurate account of the affection. Boerhaave afterward propounded a theory that nasal polyps were formed by a prolongation of the lining membrane of the pituitary sinuses. He thought that secretion in one of the cells became too thick or was otherwise unable to escape properly from the cavity and that the cavity became filled up until its lining membrane protuded from the nasal fossa.

A detailed description of the pathogenesis of the nasal polyp was given by Saint-Hilaire in 1698:

> . . . The polyp takes its origin in the osseous lamellae at the root of the nose. In order to well understand this excrescence, it is necessary to observe that the internal membrane

of the nose is very thick and spongy, and is bathed in a sticky, viscid humor, and its porosities are so arranged that it only gives passage to those parts of the blood which are thickest and most likely to produce excrescences. All these causes contribute greatly to the generation of polypi. Whenever a little heat and disturbance get into the blood, its movement increases, its viscid parts are extruded, the heat fixes them and condenses them, and their abundance in part as spongy as the nose, furnishes the substance of the polyp, because these humors becoming arrested in the tissue of this membrane, they swell its vessels and dilate its glands; the matters congeal, and are changed into a fungous and carcinomatous mass and, by the addition of new matter, the polyp enlarges and grows. The polyp indeed may also be engendered by an acrid lymph, which erodes the glands and the channels of the internal membrane of the nose in such a manner that the nutrient juice, becoming infiltrated by the ulceration of this membrane into interstices of its fibres, coagulates there and forms, little by little, those excrescences which they call polypi. One may again attribute the cause of these excrescences to the little glands of the membrane, which, in dilating, become formed together and form that which we call polypus. The acidity of the humors may, indeed, contribute to the generation of these excrescences because it can coagulate the nutrient juice, which becomes lodged in the glands, remains there, having lost its fluidity, and new juice flowing there and coagulating, it forms a tumor in the nose, which they call a polyp.

This rather involved description has purposely been included, since it demonstrates the influence of the iatrochemical and iatrophysical school of that era. In the first edition of his surgical work in 1707, Pierre Dionis, who died in 1718, made a distinction between two varieties of polyps: "One is an excrescence formed by the engorgement of the glands which line the walls of the pituitary membrane, and the other is the extension of this membrane gradually elongated."

A popular belief in lunar influences prompted Juncker to write, in 1721: "According as the moon fills or wanes, the polypi of the nose increase or decrease in size. Hence it may be concluded it is best to attack the polyp in the waning of the moon."

A more certain observation was expressed in 1742 by Gorter, who described a nasal polyp in this manner: "It seems sufficiently evident that the pituitary membrane is separated from the bone, often carious, in the cavity of the nostrils, so that it makes a pendulous sac, either single or multiple, according whether this membrane is separated from one or more depressions of the small bones, which sacs swell with secretions collected in the cellular lamellae of the membrane."

Jean Palfyn (1649-1730), however, more nearly pinpointed the origin of a polyp when he said, "A surgeon of Paris once told me he had seen a polyp which had its attachment within the cavity of the os maxillare and had grown through the hole of communication into the nose; this he had observed after death."

Lorenz Heister (1683-1758), the great German surgeon of the eighteenth century, spoke of polyps springing from the accessory sinuses and the cavities of the ethmoids as a result of obstruction of one or more glands of the pituitary membrane. In 1761 Giovanni Battista Morgagni (1682-1771) made pertinent observations that at postmortem polyps were found more frequently outside

than inside the sinuses. He also approved of Valsalva's method of removing the lamella of bone on which the polyp grows with a view to preventing recurrences.

Deschamps published a 300 page *Treatise on the Diseases of the Nasal Fossae and Their Sinuses* (Richard, Paris) in 1804. He divided nasal polyps into (1) fungous and vascular, (2) mucous and lymphatic, (3) scirrhous, and (4) sarcomatous types. His methods of treatment were (1) the local application of astringents, (2) excision with guarded bistoury, (3) avulsion with forceps, (4) the knotted thread (which he ridiculed), (5) chemical caustics and actual cautery, and (6) ligature with a waxed thread or with wire of pure silver.

Friedrich Theodor von Frerichs (1819-1885), in 1843, referred to the surface of a polyp as being similar to that of the surrounding epithelium. Max Joseph von Chelius (1794-1876), whose *Handbuch der Chirurgie* was the standard textbook in Germany until the middle of the eighteenth century, regarded the nasal polyp as a local infiltration of the mucosa with serum. Sir James Paget (1814-1899) classed nasal polyps as fibrocellular tumors. Paget's close friend, Rudolf Virchow (1821-1902), the man recognized as the founder of cellular pathology, described them as myxomas. Theodor Billroth (1829-1894), in 1855, compared the structure usually found in rectal polyps with those found in the nasal cavity and stated that nearly all of these growths were adenomatous in structure. Intimately associated with subsequent contributions to the literature concerned with the pathologic anatomy of polyps were such persons as Hopmann (1885), Emil Zuckerkandl (1882), Woakes (1885), Chiari (1887), J. H. Bryan (1889), Hajek (1896), Cordes (1893), Okada (1898), Gustav Killian (1860-1921), Uffenorde (1923), Kubo, Hirsch (1929), and Semenov (1952).

In 1872 Voltolini drew attention to the intimate connection between asthma and nasal polyps, asserting that the former disappeared when the latter were removed. He ascribed this to the reflex irritation of the mucosa propounded by Johannes Müller in 1853.

In 1881 Albert defended the method of evulsion which was attacked by specialists of that era. The following are some interestingly pertinent remarks which he made at that time:

> In late years, this method [the operation by forceps] has been condemned as brutal, painful and inefficient. There is no doubt that it can be performed brutally by rough or clumsy hands; but the conservative surgeon does not grope blindly in the nasal cavity; on the contrary, he places the patient in a proper position, makes use of a nasal speculum, and carefully selecting forceps suitable as regards size and situation of the polypus, he seizes it by the pedicle, and extracts it by gentle rotatory movements. . . . The hostility to the old universally-practiced method [evulsion by forceps] is merely the outcome of the elaborate methods used by the specialists with an object which is easy to understand.

Morell Mackenzie, whose *Manual of Diseases of the Throat and Nose* (1880, William Wood & Company, New York) contained an extensive discussion of polyps, commented as follows concerning Albert's remarks: "Specialists might perhaps retort that the hostility of some surgeons to new and improved methods

of cure, which they themselves have failed to master, has a motive which is equally easy to understand. But such amenities are better avoided in scientific discussions."

Historical documentation of the past centuries serves only to remind us how little progress has been made toward the solution of a vexing local manifestation of a constitutional disease. Inquiries have been concerned with the etiology of nasal polyps and have sought to determine whether they represent purely a nasal affection, the result of an infectious disease, an allergic condition, or a combination of both infection and allergy. With the exception of allergists, the rhinological world has continued to mimic the tales handed down by predecessors well past the turn of the century. Rhinologists have been and still are confounded by the recurrence rate of polyps, even though they now use refinements of snaring instruments instead of hippocratic methods of evulsion and extirpation, inject hydrocortisone instead of oxycrate of wine into the nose, and employ radium, x-ray, and the coagulating electrical currents in place of the antiquated caustics and cauterization techniques of the same era. Even those who center their thoughts on the physiopathology seem strikingly in tune with the prevailing thought of the Arabian hemorrhoidal theory. Nevertheless, it is essential that we continue to study present-day clinical observations as recorded in the literature with the knowledge of past thinking and the hope that soon we will get newer concepts into orbit.

Taillens[25] has attempted to summarize the mechanism of polyp formation. He ascribes nasal polyps to dilatation of arterioles, veins, and capillaries which produces an inflammatory or noninflammatory edema due to three possible factors: allergic edema in which there is a dilatation of arterioles and capillaries; an angiospastic edema in which there is a vasoconstriction of arterioles and capillaries together with a vasodilatation of veins; and a venous edema resulting from impairment of the venous circulation. This amounts to what many have repeatedly pointed out, namely, that polyps result from infection or allergy or from a combination of both. Polyps may cause the following:

(1) Pressure of edematous mucosa may obstruct ducts, and glands may become cystic (prolonged edema). Temporary edema, such as occurs during an acute rhinitis, may result in the disappearance of a polyp.

(2) Edematous mucosa may interfere with nutrition of bone, leading to decalcification and resorption of bone. Bone may evidence pressure erosion, thinning-out, or osteoporosis.

(3) Superimposed infection may cause an inflammatory reaction in the polyp or in the soft tissues of the bone.

(4) Deficient drainage and disturbed aeration or ventilation.

In the year 650, the pathologic anatomy of nasal polyps was defined by Paulus of Aegina as originating from the ethmoid cells. The controversy has raged over these 1,300 years; many are still rediscovering Paulus of Aegina. There are those, however, who contend that polyps originate from the maxillary sinus. While there are single polyps which extend from this sinus by a long

pedicle and fill the posterior choana and even dangle into the nasopharynx, in the majority of patients there are many different polyps of multisinal origin. As a matter of fact, hyperplastic changes may affect some or all of the nasal or sinal mucosa.

One factor alone seems insufficient for the production of nasal polyps. While they may be found in a patient with an allergy, an infection, or a mechanical complex (including septal deviations and spurs), polyps do not occur as the result of allergy alone; many allergic persons (with seasonal as well as perennial allergy) do not have polyps. Frankly, we do not know the etiology of polyps. Few clinicians distinguish between polyps that occur in one person and those that occur in another. This variant may explain the inconsistency in the reports of responses to therapy (both medical and surgical).

In a series of 500 cases of sinusitis, Semenov found that only 17 per cent of the patients were allergic, 47.6 per cent were nonallergic, and 35.4 per cent were equivocal or uncertain. Allergic or vasomotor rhinitis accounts for two-thirds of rhinologic complaints consisting of such symptoms and signs as (1) wet nose, (2) blotchy pallor of mucosa, (3) boggy swelling or water logging of the fibrillar stroma in which the basement membrane is thickened and hyalinized, and (4) marked tunica propria changes in the upper (subepithelial) layers wherein the tissue prolapses and becomes polypoid when it can no longer restrain edema.

Although multiple factors are involved, the reaction which results usually follows a certain pattern. First, there is a wheallike edema which distends the loose areolar stroma. Then the redundant tissue becomes flabby. The edema is due to numerous factors, such as (1) an abnormal permeability of blood capillaries, (2) venous stasis, (3) lymphatic obstruction, (4) inflammation, and (5) a biochemical disturbance.

The stages of pathologic alteration which ensue (including the inflammatory edema and exudation, the thickening and hyperplasia, and the degenerative changes producing polyps, cysts, and the like) have been well described by many of the observers mentioned previously. Division into pathologic types, such as angiomatous, cystic, glandular, and the like, has no significant meaning, as most if not all such alterations may be found in the same polyp. No objection is offered to division of the changes into stages. In the first stage there is a heavy mucoid discharge which is great in viscosity and slow in movement. This results in stagnation, and an accumulation of secretion in the sinuses is produced by the obstructive phenomenon. Histologically, we see an infiltration of lymphocytes, numerous eosinophils, and minimal mucosal thickening. In the second stage, there is definite hyperplasia with an increase in cellular elements, including mucinous epithelium, collagenous thickening, hyalinization of the basement membrane, eosinophils, lymphocytes, and plasma cells. Then, in the third stage, cysts (of both the retention and the serous variety) and polyps are in evidence.

For many years recurrence rates of polyps have interested clinicians. Jonathan Wright (1860-1928), in 1908, reviewed a number of cases of "Persistent Recurring Papillomata of the Nasal Chambers With Some Remarks on

the Differential Diagnosis of Epithelial Proliferation" and described the same problems of recurrence with which we are troubled today. This is the inverting papilloma of the nose and paranasal sinuses, a neoplastic disease which is frequently multicentric, to which members of our department have called the special attention of rhinologists. The clinical history is characterized by long-standing unilateral nasal obstruction and repeated polypectomies with subsequent recurrence of the obstructive symptoms. The nasal fossa is filled with tumor masses which usually are reddish gray and lobulated; frequently, however, they are pearly and glazed, similar to polyps. Two types of inverting papilloma are distinguished: (1) a single, cauliflower-like, sessile, or pedunculated type and (2) a multiple (so-called papillomatosis) type in which the mucosa is studded with numerous growths.

Involvement of the paranasal sinuses by either type of papilloma is not infrequent; ethmoid, antrum, and sphenoid sinuses are involved, in this order of frequency.

The histopathology of this tumor is characterized by epithelial proliferation, infolding downgrowth, crypts, and knobs. The downgrowing epithelium is stratified columnar or stratified squamous, with preservation of the basement membrane. In some cases, malignant transformation of papilloma has been observed, but, even without morphologic evidence of malignancy, this tumor may take a malignant clinical course because of its location, its tendency to recurrence, and the destruction of the osseous structures due to expanding growth.

Generally, clinicians follow a combined program to control allergic, infectious, and mechanical factors, since they are usually found in a patient with a polyp complex. Antiallergic therapy is frequently incomplete and unsatisfactory. So-called "polypectomy" is likewise inadequate and is purely symptomatic. Sinus surgery has its anatomic restrictions; the results are frequently poor, and in cases of severe allergy catastrophic responses may be produced. Etiological therapy demands a suppression of the inciting cause which, in chronic inflammation, calls for a complete cure of the diseased sinuses (for the condition is seldom confined to one sinus) and a reduction of the multiple and complex allergic factors.

Max Samter has repeatedly suggested that in certain patients the removal of polyps can encourage the development of bronchial asthma. Naturally, it must be established that polypectomy actually precipitates the response of the lower respiratory tract and that this is not simply the natural history of this particular respiratory disease. During the past eight years, in association with Samter and his colleagues, I have recorded a study of this relationship on the basis of these observations. There are two separate groups of patients, the larger number consisting of the group of atopic patients who have suffered from rhinitis and bronchial asthma since their earliest childhood and the smaller group comprising the nonatopic patients who remain symptom-free until relatively late in life, when nasal polyps and bronchial asthma develop without warning and progress rapidly.

Atopic and nonatopic patients who develop nasal polyps present, at first glance, a similar clinical picture and their complaints are almost identical. Yet, it is likely that they represent diseases of different etiology. As a matter of fact, the differences which we have observed have outnumbered the similarities. As a rule, a well-controlled atopic patient will respond to a comprehensive antiallergic regimen with marked improvement, often with complete freedom from symptoms. The elderly patient who develops nasal polyps and bronchial asthma will never respond to control, will rarely respond to treatment, and will be subject to asthmatic episodes of increasing severity. Acetylsalicylic acid relieves the atopic person, and yet it is likely to cause fatal attacks in the elderly patient with "intrinsic" respiratory disease.

For successful management of nasal polyps, we must realize that polyps may be limited in some instances to the nasal mucous membrane but that more often than not they suggest similar changes in one or all of the neighboring nasal accessory sinuses. Rhinologists have often wondered why in some patients a simple removal of polyps by snare is followed by complete alleviation of symptoms and nonrecurrence of polyps, whereas in others even repeated procedures and more extensive exenteration of the sinal mucosa produce debatable therapeutic results.

The classification proposed by Samter offers a clue as to why some patients do respond to accepted surgical procedures and others do not. Rhinologists have wavered between so-called conservative and radical intranasal and even external sinus surgery. The enthusiasm for allergy management reflects the awareness that failures from the more complete, or what Simonton calls more "comprehensive," forms of sinus surgery are not uncommon. A comparison between the effectiveness of adequate drainage and extensive surgery and the effectiveness of antiallergic management is difficult to assess in the light of the tendency to include all patients with nasal polyps in one single clinical (rhinologic or allergenic) category. The fact that the nasal and sinal involvement represents a local manifestation of a constitutional disease needs restatement as well as re-emphasis.

In treating an atopic person who suffers from seasonal and perennial rhinitis associated with extensive nasal polyps, we insist on rigid environmental control, recommend immunization to inhalants which cannot be avoided, perform a submucous resection of the nasal septum if the airways are significantly obstructed, or carry out additional surgery of the nose and paranasal sinuses as dictated by clinical and roentgenographic evidence. The results of inadequate surgery are as unsatisfactory as the results of inadequate antiallergic management.

In treating nonatopic patients, we are inclined to be conservative and to defer surgery as long as possible. It should be stated, however, that the use of corticosteroids unfortunately does not represent the final therapeutic solution for the handling of nasal polyps. Many observers agree that the symptomatic administration of hormones occasionally restores mucous membranes to seemingly normal appearance and function but that, upon withdrawal, nasal polyps inevitably reappear.

Contrasting methodology characterizes the surgical management of polyps. In rhinology, extremes are cyclic; there are periods of encouragement of non-surgical methods (including control of allergy and treatment of associated sinus infections by displacement and direct sinus irrigation) and of extensive or "comprehensive" external or intranasal surgical procedures aimed at exposing and rendering free the polyp-bearing mucosal lining of every accessible sinus area. There are those who have been discouraged by all approaches and are content to resort to the snaring of polyps which become large enough to interfere with nasal respiration.

Vasomotor rhinitis was recognized and discussed extensively by Bosworth (1843-1926) in 1889. It continues to be the most common cause of nasal blockage, and continuation of it brings about mulberry (hypertrophy) changes in the posterior ends of the inferior turbinates. This is a papillary-like excrescence in which mucous glands are abundant. Many clinicians believe that the nose acts as the "barometer of our emotions" and therefore deem vasomotor rhinitis to be of functional or psychosomatic origin. These same observers are of the opinion that continued vasomotor rhinitis may lead to polyps, and they consider psychotherapy prominently in the management of recurrent polyps.

In diagnosis, as well as in management, consideration must be given to other types of tumors which may have polyps superimposed upon them. In most instances the presenting mass in the nose consists of polypoid tissue (especially if the mass is unilateral), and considerable care must be exercised in the investigation so that the condition is not disposed of as representing "merely nasal polyps." Of course, the need for biopsy is evident.

The underlying tumor, even though histologically benign, such as chondroma, fibroma, mixed tumor, angiofibroma, neurofibroma, hemangioma, osteoma, and inverting papilloma, may have such a clinical course of expansion and recurrence as to endanger the orbital and intracranial structures. Wide exposure by means of an external incision (Weber-Fergusson) is advocated to ensure adequate removal and thus prevent recurrence.

In contemplating the problems posed by recurring nasal polyps, we should consider also the complications engendered by blockage of the natural openings of the sinus which may produce mucoceles; the osteitis and osteomyelitis brought about by infection and the interference with the nutrition of the soft tissues of bone (periosteum, marrow, and endosteum).

SUMMARY

The ancients were well aware of the presence of nasal polyps and of the problems which they presented, and they devised ingenious methods for their removal.

The evaluation of the present knowledge of the etiology and treatment of nasal polyps must be viewed with a degree of skepticism engendered by a lack of unanimity of opinion.

We do not now know the etiology of polyps. Polyps do not occur solely as the result of allergy; many allergic patients (with seasonal and perennial

allergy) do not have them. Polyps represent a hyperplastic change of one portion or all of the nasal or sinal mucosa.

Clinicians fail to distinguish between polyps that occur in one person and those that occur in another. This might explain why the same management or procedure is ineffective in one patient and effective in another.

A patient with nasal polyps must be distinguished by an additional factor, possibly an inherited structural deficiency of connective tissue.

Atopic patients with seasonal and perennial rhinitis associated with extensive nasal polyps may undergo surgery of the nose and/or sinuses. Nonatopic persons, however, are best approached with surgical reluctance, since bronchial asthma develops rapidly in this group.

Dissatisfaction with present methods of management arises from failure to control the regrowth of polyps. This applies to those patients whose rate of recurrence of polyps has been prolific and rampant.

Attention is directed to the fact that polyps may be a presenting part of underlying tumors, thereby clouding the true nature of the growth pattern. Furthermore, the proliferative nature of polyps may indicate a type of pathologic entity termed "inverting papilloma," erroneously considered to be "merely polyps."

An integrated study by the allergist and the rhinologist should be encouraged if the problem is to be solved.

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PURIFICATION OF ANTIBODIES. I. DEXTRANASE PURIFICATION OF ANTIBODY PRECIPITATED FROM TYPE II ANTIPNEUMOCOCCAL RABBIT SERUM BY DEXTRAN

Introductory Report

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INTRODUCTION

CAMPBELL and Lanni,[1] in a discussion of the purification of antibodies, stated: "It is becoming increasingly evident that many types of investigations of the fundamental nature of antibodies require purified preparations for interpretable results. It is important therefore that better methods be demonstrated for the isolation of purified antibodies." In this laboratory efforts to identify the antibody which mediates specific anaphylactic passive desensitization[2] have emphasized this need for purified antibodies. So far, all antisera which have mediated specific passive desensitization have precipitated strongly with appropriate antigens or haptens.

It seemed logical, therefore, to attempt to determine whether precipitin was the antibody which prevented anaphylaxis specifically. The obvious approach to this problem was to purify precipitated precipitin. The experiments to be described were suggested by two reports in the literature, after several unsuccessful attempts to purify precipitated precipitin had been made. In one report, Hehre and Sugg[3] showed that dextran formed specific precipitates with Types II, XII, and XX antipneumococcal sera. In the other report Tsuchiya and colleagues[4] reported a study of the enzyme, dextranase, which degraded dextran. The use of dextranase to purify antibody which had been precipitated from Type II antipneumococcal rabbit serum by dextran was the natural sequel to those reports.

This is believed to be the first demonstration of the successful purification of precipitated antibody by enzymatic elution of the precipitating hapten.

EXPERIMENTAL DATA

Preliminary experiments showed that (1) dextran formed a heavy precipitate with Type II antipneumococcal rabbit serum, (2) guinea pigs sensitized

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These studies were made possible by Grant #1306-E from the National Institute of Allergy and Infectious Diseases.

We are grateful to The Lederle Laboratories of the American Cyanamide Corp., Pearl River, New York, for the polysaccharide and to Wyeth Laboratories, Marietta, Pennsylvania, for the antipneumococcal rabbit serum used in these experiments.

Received for publication Feb. 11, 1959.

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passively with 0.2 ml. of Type II antiserum died promptly in anaphylactic shock when challenged with 0.5 mg. of dextran intravenously, (3) dextranase,* in small amounts, denatured aqueous solutions of dextran effectively at pH 5.0 in 2.5 hours at 37° C. (dextran was considered denatured when the solution no longer caused a precipitate when added to the antiserum), and (4) Type II antiserum still precipitated strongly with dextran and with Type II polysaccharide after incubation with dextranase for 2.5 hours at 37° C. and pH 5.0.

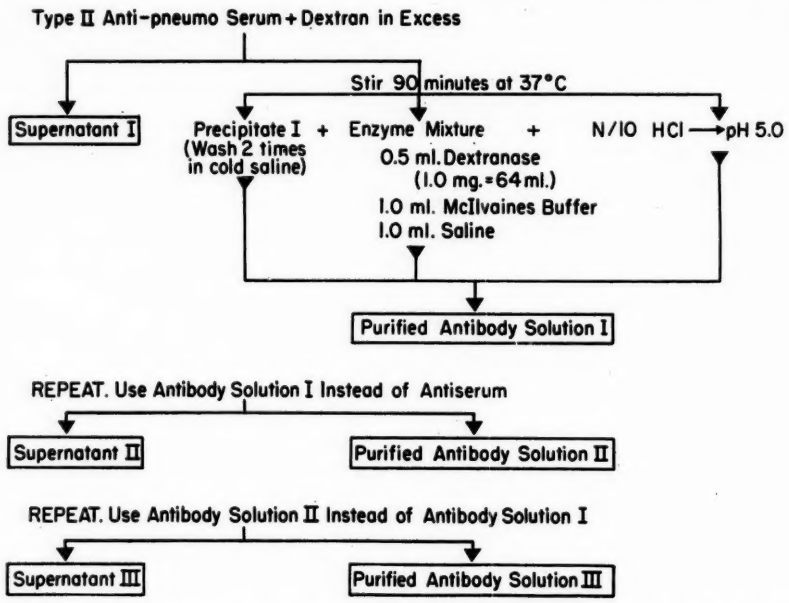
Type II Anti-pneumo Serum + Dextran in Excess
Stir 90 minutes at 37°C
Supernatant I
Precipitate I (Wash 2 times in cold saline)
+
Enzyme Mixture
0.5 ml. Dextranase (1.0 mg. = 64 ml.)
1.0 ml. McIlvaines Buffer
1.0 ml. Saline
+
N/10 HCl → pH 5.0
Purified Antibody Solution I
REPEAT. Use Antibody Solution I Instead of Antiserum
Supernatant II
Purified Antibody Solution II
REPEAT. Use Antibody Solution II Instead of Antibody Solution I
Supernatant III
Purified Antibody Solution III

Fig. 1.—Purification of antidextran antibodies with dextranase. Outline of method.

PURIFICATION OF ANTIBODIES (FIG. 1)

First Purification.—Dextran, 6.0 mg. was added to 30.0 ml. of anti-pneumococcal Type II rabbit serum. The supernatant gave a positive precipitin ring test with Type II antiserum. Three additional aliquots of 3.0 mg. of dextran caused no further precipitation. After centrifugation, the supernatant was decanted and stored in the icebox. The precipitate, 0.6 ml., was translucent, serum-colored, and firm. The precipitate (No. 1) was washed with cold distilled water and then with cold saline.

The washed precipitate was suspended in a mixture of 1.0 ml. saline, 1.0 ml. McIlvaine's buffer,[11] and 0.5 ml. of dextranase (1.0 mg./64 ml.). This suspension was adjusted to pH 5.0 with 0.1 N hydrochloric acid and stirred mechanically at 37° C. for ninety minutes, at which time all of the precipitate had dissolved. This solution was made up to 15.0 ml. with normal saline, adjusted to pH 8.0, and traces of debris were removed by centrifugation.

*A mixture of dextranases #3553-69 obtained from *Penicillium funiculosum* NRRL 1768 in the Northern Utilization Research Branch of the United States Department of Agriculture, Peoria, Illinois, under the direction of Drs. H. M. Tsuchiya and R. G. Benedict. We are grateful to them for sharing their limited supply with us. It contained 870.4 dextran units per milligram when they measured it against NRRL B-512 F dextran, also donated by Drs. Tsuchiya and Benedict.

This purified antibody solution No. 1 gave negative precipitin (ring) tests with Type II antiserum, a nonspecific antiserum, saline, Merthiolate, and a nonspecific antigen. In other words, the purified antibody had no demonstrable dextran. It precipitated specifically with dextran.

Second Purification.—Dextran was added to 10.0 ml. of purified antibody solution No. 1 in four aliquots of 5.0, 5.0, 1.0, and 1.0 mg. in a total of 1.2 ml. of saline. The supernatant was decanted after centrifugation. This contained an excess of dextran, as shown by the supernatant's failure to precipitate with dextran after the fourth addition, whereas the Type II antiserum gave a strongly positive precipitin test. The precipitate No. 2 was whitish, firm, slightly translucent, and no longer serum-colored. The volume of precipitate obtained per milliliter of purified antibody solution was grossly the same as that obtained from the antiserum. This precipitate No. 2 was purified in the same way as precipitate No. 1. Purified antibody solution No. 2 was made up to the original volume of 10.0 ml. with saline and adjusted to pH 8.0. It precipitated strongly with dextran but did not precipitate with Type II antiserum, indicating that it had been cleared of dextran.

Third Purification.—Dextran, 5.0 mg. in 0.5 ml. of saline, was added to 5.0 ml. of the purified antibody solution No. 2; 0.15 ml. of precipitate No. 3 formed. This precipitate had the same appearance as precipitate No. 2. The supernatant precipitated strongly with Type II antiserum but not with dextran, indicating a dextran excess.

Precipitate No. 3 was purified in the same manner as the two preceding precipitates. It was then made up to 5.0 ml. with saline and adjusted to pH 8.0. The purified antibody solution No. 3 precipitated with dextran but not with Type II antiserum, indicating that it had been cleared of dextran.

Purification of Supernatants.—Supernatants 1, 2, and 3 were decanted from the precipitates and purified (freed of the dextran excess) by the addition of 0.5 to 1.0 ml. of dextranase buffered at pH 5.0 and by stirring at 37° C. for seventy-five to ninety minutes. Each of the three purified supernatants precipitated strongly with Type II antiserum before, but not after, purification. The additional observation was made that the supernatants did not precipitate with dextran before or after purification, indicating that dextran had precipitated all of its specific precipitin on each occasion. The supernatants did, however, precipitate with the Type II polysaccharide.

ADDITIONAL EXPERIMENTS WITH THE PURIFIED ANTIBODY SOLUTIONS

Sensitizing Capacity.—Six guinea pigs were sensitized passively in pairs. Each pair was given intraperitoneal injections of 0.04 to 1.0 ml. of purified antibody solution I, II, or III, respectively. One milliliter of antibody solution contained the rough equivalent of antidextran precipitin obtained from 2.0 ml. of the Type II antiserum. All animals were challenged intravenously the next day with 2.0 mg. of dextran. Both of the pigs which had been sensitized with purified antibody solution No. 1 died promptly in anaphylactic shock. One of those which had been sensitized with antibody solution No. 2 died promptly.

The other had a moderately severe anaphylactic reaction but recovered. One of the pigs sensitized with antibody solution No. 3 had a moderately severe reaction, and the other died promptly in anaphylactic shock. Fifteen other pigs had been sensitized and shocked with varying amounts of the antibody solutions and dextran in preliminary experiments.

Persistence of the Dominant Hapten Phenomenon in Purified Antibody Solution No. 3.—Type II antiserum is known to precipitate with dextran and with its specific polysaccharide. Dextran was added in small aliquots to a small portion of purified antibody solution No. 3 until there was no more precipitation and until a portion of the supernatant precipitated strongly with fresh antibody solution, indicating dextran excess. Type II pneumococcal polysaccharide precipitated strongly when added to the rest of the supernatant. Another portion of the antibody solution was treated with similar aliquots of the polysaccharide instead of dextran. A sample of the supernatant precipitated strongly when fresh Type II antiserum was added to a small portion of it, indicating polysaccharide excess. The rest of the supernatant did not precipitate when dextran was added. In other words, the polysaccharide precipitated all of the antidextran and antipolysaccharide antibodies, but dextran precipitated only the antidextran antibody. This is another example of the dominant hapten role of polysaccharide and the recessive role of dextran in the Type II antipneumococcal system[9] (see discussion).

Miscellaneous Observations.—Dextranase is effective over wide temperature and pH ranges. For example, dextran antibody precipitates were left in the icebox at pH 7 and pH 8 for five days. The dextran had been degraded, and the antibody was in solution at the end of that time. Dextranase did not elute the dextran when mixtures of dextranase-dextran-antibody precipitates were kept frozen for five days. There was little gross attrition in the packed volume of the precipitates during the successive purifications and reprecipitations.

DISCUSSION

Similar attempts to purify precipitated antibodies with enzymes will be limited, obviously, to those immune systems which employ antigens or haptens for which specific degrading enzymes are known or can be developed. This method has the advantages of simplicity and of a high yield of virtually unlimited amounts of seemingly pure antibody. This approach to the problem of purification of precipitated antibodies should not be confused with the purification of diphtheria antitoxic sera by proteolytic enzymes,[5, 7] the result of which is reduced antigenicity and concentration of the toxin-neutralizing antibodies.

Campbell and his associates[1, 5] and others[8, 12] have called attention to the limitations of the methods of purifying precipitated antibody which employ precipitation of globulin by salts, alcohol, temperature, and pH variations, dialysis, electrophoresis, convection, dissociation of antigen-antibody complexes,

etc. Of these methods, two approximated 100 per cent yield of almost pure antibody. One[6] utilized the principle of elution of antibody which had been absorbed by an insoluble antigen-cellulose complex. The other[1] employed dissociation of an antigen-antibody complex in which the antigen was insoluble in acid.

The antipneumococcal Type II-dextran system employed here should provide a useful model with which to study the mechanics of hapten precipitation and the phenomenon of dominant and recessive haptens.[9] In the latter phenomenon, first observed during these experiments, pneumococcal polysaccharide Type II still (a) precipitated with Type II antiserum after all antidextran precipitins had been removed and (b) elicited anaphylactic shock in passively sensitized guinea pigs which had been desensitized actively with dextran. The reverse was not true. In other words, the polysaccharide removed all antidextran precipitins and actively desensitized against dextran.

This antipneumococcal-dextran system may serve also as a model for the study of antibody valence. The question is raised by these experiments as to whether (a) the antibodies which precipitated with polysaccharide and with dextran were different or (b) whether the same globulin molecule was capable of precipitating with both haptens. The dominant hapten phenomenon and the presence of antipolysaccharide precipitins in the third purification of the precipitates suggest the latter. On the other hand, the answer to the question might be found in prosthetic groupings common to both haptens.

Persistence of the association of specific sensitizing and precipitating functions in the purified antibody solutions makes it necessary to reappraise the experiments of Kabat and Benacerraf[10] which implied that the anti-ovalbumin sensitizing and precipitating antibodies had been separated. They did not examine their precipitates for sensitizing antibody. This problem is being studied.

The following experiments are in process: (1) tests of the capacity of purified antidextran solutions to confer specific desensitization, the original aim of these experiments, and (2) more detailed studies of the immunologic behavior of the several supernatants, washings, and purified antibody solutions.

SUMMARY

1. Dextran precipitated sensitizing and precipitating antibodies from Type II antipneumococcal rabbit serum. All detectable antidextran precipitins were precipitated by dextran in the zone of hapten excess.

2. Dextran elicited anaphylactic shock in guinea pigs that had been sensitized passively with Type II antipneumococcal rabbit serum.

3. Dextranase denatured dextran in saline and in protein solutions and in insoluble complexes with precipitated antibody.

4. Dextranase eluted all readily detectable dextran from dextran-antidextran precipitates. The resulting "purified" antibody precipitated with and passively sensitized guinea pigs against dextran.

5. Antibody which had been purified three times still sensitized against and precipitated with dextran.

6. Purified antibody solutions still exhibited the phenomenon of dominant and recessive haptens.[9]

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THE USE OF GAMMA GLOBULIN IN THE TREATMENT OF INTRINSIC ASTHMA

Report by Committee on Therapy, American Academy of Allergy, Milwaukee, Wisconsin

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PURPOSE

THE current group study was initiated in an effort to determine whether or not gamma globulin therapy had a place in the management of intrinsic asthma. Recent studies by Favour[1] and by Abernathy and associates[2] would suggest that it has no value. Favour's study involved a small group of patients, primarily adults; Abernathy's group, although composed of a larger series of children, was similarly small. Under the circumstances, we felt that further study on a broader scale was indicated.

METHOD

In order to collect a relatively large series of any significance, uniformity of case material as well as methodology was essential. A group of physicians cooperated, and only those reports meeting certain criteria were used in the study.

All patients were children under the age of 12 who had asthma definitely associated with infection. In each case routine allergy care, as well as drug therapy and antibiotics, was used and stabilized. Then, when asthma precipitated by infection ensued, the gamma globulin was given in doses of 0.5 c.c. per kilogram of body weight. The dose was repeated every two weeks for three injections before a decision concerning lack of effect on the course of asthma was reached. If benefit ensued, the reporting physician estimated the benefit obtained. Blood gamma globulin determinations were made electrophoretically or chemically prior to and just after the end of gamma globulin therapy.

RESULTS

A total of eighty-four satisfactory cases were accumulated, and as shown in Table IV, about one-half of the patients obtained some benefit. A breakdown of the group according to age is of interest. Patients under the age of 1 year

Gamma globulin for this study was supplied by the American Red Cross.

The following physicians collaborated in the case studies: Drs. Earl B. Brown, New York, New York, S. J. Levin, Detroit, Michigan, and Roland Scott, Washington, D. C.

Received for publication Feb. 19, 1959.

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TABLE I. UNDER 1 YEAR OF AGE

Post-gamma globulin level higher—3 patients had 50-75% benefit	
Pre-gamma globulin low	
Post-gamma globulin level same—1 patient had less than 50% benefit	
Pre-gamma globulin normal*	1 patient had 50-75% benefit 1 patient had less than 50% benefit

*A level of 0.6 Gm. per cent or better was considered normal.

(Table I), a group in which a lower-than-normal blood gamma globulin level is not too unusual, showed, in four out of six cases, 50 per cent or more amelioration of asthma. Three of the four patients obtaining benefit had low initial gamma globulin levels, and all three sustained a definite rise in the blood level following therapy. One with an initially low level and no post-therapy change

TABLE II. AGES 2 THROUGH 5 YEARS

	RELIEF OF ASTHMA			
	0	LESS THAN 50%	50-75%	MORE THAN 75%
Pre-gamma globulin low				
Post-R gamma globulin up		2	2	
Post-R gamma globulin same		1	1	
Pre-gamma globulin normal	5	5	4	3
Level not done	4	2	2	1

showed no improvement. In the group from 2 to 5 years of age (Table II) the results showed nineteen children with no or slight (less than 50 per cent) benefit and thirteen with moderate to marked benefit. Table II does not bear out the benefit to children with low gamma globulin levels suggested by Table I. Of

TABLE III. AGES 6 THROUGH 12 YEARS (NONE WITH LOW GAMMA GLOBULIN)

	BENEFIT TO ASTHMA			
	0	LESS THAN 50%	50-75%	MORE THAN 75%
Gamma globulin normal	9	7	11	10
Gamma globulin not done		4	3	2

the older children, aged 6 through 12 years, none were reported to have low gamma globulin levels. Here twenty children had little or no benefit, compared to twenty-six who had good results. The final total of all groups reveals that 51 per cent had some good results. Of this total, only 19 per cent had more than 75 per cent benefit. The bulk of cases (49 per cent) had little or no change in the asthmatic pattern.

TABLE IV. TOTALS OF ALL GROUPS

BENEFIT				TOTAL
0	LESS THAN 50%	50-75%	75% OR MORE	
18 (22%)	23 (27%)	27 (32%)	16 (19%)	84

It would appear that further study on the use of gamma globulin in patients with low blood levels, particularly those under the age of 1 year, in whom this situation is prone to occur, should be made. The present group in this age area is too small to be conclusive. Otherwise, gamma globulin does not appear to affect the course of infectious asthma materially, although some clinical improvement did occur in most cases.

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DELAYED HYPERSENSITIVITY IN SEVERAL STRAINS OF MICE STUDIED WITH SIX DIFFERENT TESTS

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PUBLISHED evidence accumulating from widely separated laboratories indicates that mice can develop delayed hypersensitivity (tuberculin allergy) which can be detected by several methods and distinguished from simultaneously occurring immediate hypersensitivity.[1] However, detecting tuberculin allergy seems to have proved more difficult in mice than in the larger laboratory animals. Different workers using the same test sometimes have reported conflicting results. For example, in one laboratory[2] mice shown to be hypersensitive to tuberculin by delayed systemic reaction have not reacted to this material injected into their footpads, whereas this same footpad test has been the mainstay of delayed hypersensitivity research in another laboratory.[3, 4] Strangely, mice shown by other tests to be allergic to tuberculin have not reacted to intracutaneously injected tuberculin as do other animals and man.[2, 5-7] Although, with present information, adequate explanations for these apparently contradictory results cannot be offered, one might suppose that they could be due partially to slight variations in the same technique applied in different laboratories. Differences in the mouse strains employed also might be important. Other reasons are likely to be found as more is learned about this type of allergy in mice.

Examination of the characteristics and mechanisms of tuberculin allergy in mice also should reveal information that is useful and, indeed, indispensable to workers in various fields of biology who use mice. For example, tissue homograft rejection reactions probably are due to delayed hypersensitivity.[8, 9] Yet, although mice are common subjects for homograft experimentation, their users are unable to employ the concept of delayed hypersensitivity freely in their thinking, since mice generally are thought incapable of developing it or are suspected of reacting in a manner fundamentally different from that seen in other commonly used laboratory animals.

The purpose of the experiments reported here was to compare for efficiency and practicality under similar conditions several techniques which can be used to detect delayed hypersensitivity in mice, to utilize some of these for comparing the degree and frequency with which several commonly used

From the Colorado Foundation for Research in Tuberculosis and the University of Colorado School of Medicine.

Supported by National Science Foundation Grant G 4025 and United States Department of Health, Education, and Welfare Grant E-2009.

Received for publication Feb. 25, 1959.

inbred mouse strains can develop delayed hypersensitivity, and to acquire information on certain aspects of mouse tuberculin allergy which might help explain what have seemed to be paradoxical findings, such as the fact that mice allegedly fail to react to tuberculin injected intradermally.

MATERIALS AND METHODS

C57BL, C57BR, C57L, C3H, DBA, and Swiss Webster (S.W.) mice from Rockland Farms (New York) and CF#1 mice from Carworth Farms (New York) were purchased when they were about 6 weeks old. Rockland Farms mice were all females, whereas there were about equal numbers of male and female CF#1 mice. These animals, kept in small groups, were supplied liberally with Rockland mouse diet and water.

Tubercle bacilli were grown on Kirchner medium with sodium glutamate instead of asparagine and supplemented with cubed potato. Transfers were made frequently so that bacillary suspensions could be made with actively growing pellicle. Two strains of nonpathogenic tubercle bacilli, H37Ra and BCG, were employed.

Tuberculoprotein antigen was purified from filter-sterilized, unsupplemented Kirchner medium which had supported growth of a human variety of virulent tubercle bacilli. This protein was precipitated once with ammonium sulfate, and for the systemic test it was purified by three precipitations with trichloroacetic acid from buffered solutions.[10] After being dialyzed against distilled water, it was lyophilized and stored in a refrigerator until used.

Water-in-oil (w/o) emulsions were employed for vaccinations. Ordinarily, these emulsions are produced by mixing the aqueous phase vigorously with light mineral oil (paraffin oil) containing a small amount of surface-active emulsifier. Since light mineral oil is an ill-defined mixture of saturated hydrocarbons whose varying composition may affect the reproducibility of immunology experiments,[11] it was replaced by n-hexadecane (Distillation Products, Inc., Rochester, New York), a single saturated hydrocarbon of similar physical properties. This fluid's use was suggested by the work of Hoyt and coworkers[12] in which, however, it was not employed in stable w/o emulsion. Our emulsion consisted of physiologic sodium phosphate buffer (pH 7.4), n-hexadecane, and Arlacel A (mannide mono-oleate, generously supplied by the Atlas Powder Company, Wilmington, Delaware) in the respective proportions of 10:4:1. When it included tubercle bacilli, these were suspended thoroughly in the buffer with a fine-grind Ten Broeck tissue grinder before emulsification.

Unless otherwise indicated, "test" mice were sensitized with two subcutaneous injections of 0.1 ml. w/o emulsion containing 0.5 mg. of bacilli. The injections were applied one week apart in opposite groins. Unsensitized "control" mice received similar injections of emulsion without antigen. Generally, the mice were challenged with either a bacillary suspension or a solution of tuberculoprotein about three weeks after the first vaccination. To make one of these suspensions, freshly harvested living bacillary pellicle was

blotted and weighed and then thoroughly dispersed in pH 7.4 physiologic sodium phosphate buffer with a Ten Broeck tissue grinder. Tuberculoprotein was dissolved to the required concentration in the same kind of buffer.

One or more of six different tests for delayed hypersensitivity were applied to the various strains of mice. Two of these, the footpad and skin tests, will be described in detail since they have not seen wide enough use in mice to be familiar to most laboratory workers and since their successful implementation in delayed hypersensitivity experiments seems to depend upon careful attention to technical details. The others will be described only briefly.

Since different kinds of tests were employed, results have had to be recorded in several different units of measurement. However, since mouse strains and the tests applied to them are irreducible variables, it is essential, at the expense of presenting detailed quantitative data, to report most of these results in common form (reaction or no reaction) so as to avoid confusion. Therefore, the way in which reactions were judged is explained along with the technique of the test which evoked them.

Sensitized mice that are challenged with bacillary suspensions intravenously or intraperitoneally undergo delayed hypersensitivity reactions signaled by lung density increases.[1] The technique for measuring these increases as objective indicators of both frequency and degree of sensitization in test mice as contrasted to control mice has been detailed previously.[1] It consists of removing the lungs forty-eight hours after challenge and determining their density or specific gravity by noting in which of a series of organic solvent baths, increasing regularly in specific gravity, the lungs float. According to this test, a mouse was considered to react when its specific gravity equaled or exceeded 0.71, for lungs in unsensitized challenged mice rarely reach this value unless the challenge suspension itself is unusually toxic.

It was possible to induce systemic tuberculin reactions in test mice by injecting the animals intravenously with tuberculoprotein or tubercle bacilli. With the group to which the mice being observed belonged remaining unknown, these reactions were judged on an arbitrary 0 to 4 scale according to severity of tuberculin shock symptoms evident twenty-four and forty-eight hours following challenge, that is, ruffled fur, irregular breathing, closed eyes, lethargy, and lack of irritability.[2, 13-17] Mice classified as reactors had to show at least ruffled fur and irregular breathing, neither of which is seen in normal mice.

Systemic tuberculin reactions cause body temperatures to vary.[13-20] In order that these variations might be followed in a mouse challenged intravenously, the glycerine-lubricated bulb of a rectal clinical thermometer was held in the animal's rectum for one minute. Before challenge, temperatures of all mice in a group were determined several times to within 0.1° C. to establish a normal mean, and then they were determined at various intervals after challenge. A variation of 2° C. from the established unchallenged group mean was considered to indicate a reaction, although not necessarily

one due to delayed hypersensitivity, as will be seen below. The statistical probability of such a variation in the mean of a normal group of mice was less than 0.001.

The intramuscular test of Selbie and O'Grady[6] was employed unchanged except for challenge antigen as indicated below. However, whereas these workers read reactions as increases in mediolateral test thigh diameter, we have recorded them as differences in circumference between the thigh tested and the untested opposite member. In readings made repeatedly on normal mice as well as on test mice, chance 2 mm. variations of thigh circumference occurred with a frequency of less than 0.07. Hence, mice were recorded as having reacted to a challenge if they developed this much difference between test and control thighs.

As mentioned earlier, some difficulty has been reported with the footpad test,[2] and the intracutaneous test never has succeeded.[2, 5-7] Neither test is as simple to perform in mice as in larger animals; consequently, the fact that both have failed may be due largely to technical difficulties. Their detailed description, then, is called for even though occasionally it may duplicate directions given by others.

This laboratory's footpad test technique is nearly the same as that employed by Gray and Jennings.[3] Approximately 0.015 ml. of bacillary suspension was injected into the sole of one of the rear feet with a tuberculin syringe and a 27 gauge needle whose bevel had been filed to one-half its original length and then resharpened. The foot of the mouse was drawn through the end-plate arch of a mouse-holder ordinarily meant for the tail, and its toes were flexed backward sharply by the left thumb and index finger to tauten the sole. Then the needle was inserted slowly, usually with a slight twisting motion and with the bevel up and nearly parallel to the sole, through the middle of one of the two median footpads and beyond it by about one bevel length into the sole, and the suspension was injected. In our experience, if the bevel is within a footpad itself almost no fluid can be injected, but if it is inserted into the sole injection is easy. If the needle is thrust too deeply, the suspension will be deposited subcutaneously; in that event, it does not form a localized bleb but instead can be seen to creep up the leg. Puncturing one of the prominent superficial veins should be avoided.

Reading footpad reactions was quite simple, as the feet of reactors had become very obviously reddened and swollen twenty-four and forty-eight hours after challenge. Although grading reaction intensity (on a 0 to 4 scale) was subjective, differentiating between reactors and nonreactors could hardly be considered so.

Mouse skin is very thin. Hence, injecting it is not easy without some experience and without a sharp 30 gauge needle. Moreover, the skin's thinness makes a subcutaneous injection resemble an intracutaneous one. Although volumes injected intradermally need not necessarily decrease in proportion to animal size (the same volume commonly is used in guinea pigs as in man), they could be somewhat better fitted to the thinness of the skin

being injected than is suggested in published reports on mouse tuberculin tests. In these,[2, 5, 21] 0.1 ml. has been used just as it is in guinea pigs. It would seem that to use proportionately such a large volume when the same quantity of antigen could be dissolved in one-tenth as much unnecessarily risks excessive injection trauma as well as antigen leakage into subcutaneous tissues, with the possibility of confusing local and systemic physiologic complications. Therefore, in the present experiments approximately 0.01 ml. of 1 per cent tuberculoprotein was the skin-test volume used.

Following induction of light anesthesia with 1 mg. of secobarbital sodium (Lilly) injected intraperitoneally, each mouse was immobilized on a glass plate with two strips of masking tape laid parallel, one across the side of the thorax and the other across the side of the pelvis in such a way as to tense the flank to be injected. Next, hair in this area was clipped and shaved off with a sharp razor blade, and the injection was made in the center of the flank with the needle aimed toward the head. The 30 gauge needle was inserted, bevel up, into the skin at the shallowest possible angle slightly beyond the length of its bevel. Injecting 0.01 ml. caused formation of a bleb about 1 mm. high and 2 mm. in diameter. At first, injections were performed under ten-power stereoscopic magnification, but later, when sufficient skill had been developed, they were done by observation with a 2.75-power stereoscopic headband magnifier which offered greater mobility and depth of focus. In order to evade skin injuries which can complicate reaction readings, a sharp razor must be utilized for shaving the skin, and the 30 gauge needle should be replaced at the first sign that it is becoming dull.

Induration diameter was our primary measure of reaction, since sensitized mice usually developed areas of induration 3 mm. or more in diameter twenty-four and particularly forty-eight hours after challenge, whereas in unsensitized mice an area greater than 1 mm. was uncommon. This numerical criterion for reaction is arbitrary and would not necessarily apply to experiments in which another batch or quantity of protein challenge antigen of potentially different primary toxicity was used.

EXPERIMENTS AND RESULTS

In the first experiments, four strains of mice (C57BL, C3H, DBA, and CF#1) were tested for tuberculin allergy by four different techniques. The mice of each strain were divided into test and control groups of ten mice each for lung density tests and five each for the other tests, and then they were vaccinated as indicated previously. The most significant data pertaining to this experiment are presented in Table I. For simplicity, for comparison with results published elsewhere on delayed hypersensitivity in general, and because the lung density test routinely has been done forty-eight hours after challenge, only forty-eight-hour readings are shown. Basically, forty-eight-hour and twenty-four-hour readings have been similar, although the latter sometimes have been more informative when weakly sensitized mice were being tested, and they are used frequently below. For reasons mentioned previously, results are given in terms of proportions of reactors.

Both primary tests (lung density and footpad) clearly indicate that all four mouse strains developed delayed hypersensitivity, the C3H mice perhaps less frequently than C57BL, DBA, and CF#1 mice. Although not shown in Table I, reaction intensity among reactors essentially was the same. The

TABLE I. PER CENT OF MICE OF DIFFERENT STRAINS REACTING TO FOUR TESTS FOR DELAYED HYPERSENSITIVITY*

TEST†	CHALLENGE	MOUSE GROUP AND STRAIN							
		TEST				CONTROL			
		C57BL	C3H	DBA	CF#1	C57BL	C3H	DBA	CF#1
Lung density	0.03 mg. H37Ra intravenously	ND	ND	89	100	ND	ND	0	14
Lung density	0.03 mg. H37Ra intraperitoneally	56	37	ND	ND	10	10	ND	ND
Footpad	0.015 mg. H37Ra	100	60	80	74	0	0	0	0
Intramuscular	0.03 mg. BCG	20	60	80	100	20	20	0	0
Footpad	0.015 mg. H37Ra	100	60	80	33	17	0	0	0
Intracutaneous	0.01% protein	60	67	100	100	0	40	0	67

ND = Not done.

*Readings taken forty-eight hours after challenge.

†The first three tests performed twenty-four days and the last three thirty-two, thirty-eight, and forty-seven days after the first vaccination.

apparently fewer C57BL and C3H mice reacting according to the lung density test may be spurious and due to the necessity, because their skins were so heavily pigmented, of challenging these mice intraperitoneally instead of by the considerably more efficient intravenous route.

Results from the secondary tests (intramuscular, second footpad, and intracutaneous) performed on mice of the footpad group above agree generally with those of the primary tests. Bovine variety tubercle bacilli (BCG) were used for intramuscular challenge to determine whether the cross-reactivity of mice sensitized with a human variety of bacilli was of about the same degree as occurs in other animal species.[22] In C3H, DBA, and CF#1 mice it was about the same, but C57BL mice seem to have cross-reacted rather poorly. Whether this truly is a mouse strain characteristic remains to be investigated.

Occasionally in each of the secondary tests some control mice may have reacted. Although it is possible that they were beginning to develop hypersensitivity in response to previous repeated challenges with entire bacilli by the time the intracutaneous tests were done, reactions to the intramuscular and second footpad tests probably were nonspecific and due to injection trauma or to the toxicity of challenge antigens, particularly in view of their irregularity.

Evidence is available, both from other laboratories and from this one, that the lung density, footpad, and intramuscular tests detect tuberculin allergy.[1] Such grounds for the skin test have not yet been published, however, and indeed its success in this experiment was unexpected. Nevertheless, numerous supporting experiments following this one have verified it and have indicated that the reactions measured truly were of the delayed type. Descriptions of several of these experiments will be published later, but some are presented below by way of illustration.

A second experiment resembling that just described but using three new mouse strains was set up. Two strains, C57BR and C57L, were pigmented; the third, Swiss Webster albino, was included as one well known and widely used. These mice were grouped and vaccinated as in the preceding experiment, except that each group consisted of ten animals. The two primary tests were done twenty-one days after the first vaccination, and the secondary test was applied to an evenly distributed mixture of mice from each of the primary tests seven days later. Data are presented in Table II and Fig. 1.

TABLE II. PER CENT OF MICE OF DIFFERENT STRAINS REACTING TO THREE TESTS FOR DELAYED HYPERSENSITIVITY*

TEST†	CHALLENGE	MOUSE GROUP AND STRAIN					
		TEST			CONTROL		
		S.W.	C57BR	C57L	S.W.	C57BR	C57L
Intramuscular	0.03 mg. H37Ra	100	63	100	0	0	0
Footpad	0.015 mg. H37Ra	90	100	78	0	10	0
Systemic	2 mg. tuberculoprotein intraperitoneally	55	50	88	0	0	0

*Readings taken twenty-four hours after challenge.

†Intramuscular and footpad tests performed twenty-one days and systemic tests performed twenty-eight days after vaccination.

The results indicate that no mouse strain differed particularly from another in frequency of hypersensitization. However, Swiss Webster mice seem to have reacted more intensely than those of the other two strains. Thus, as Fig. 1 shows particularly for the twenty-four-hour reading, it was in these mice that the objectively measured intramuscular tests were the most intense. Moreover, their 8.6 mm. twenty-four-hour thigh swelling exceeds that seen in any of the mice in the first experiment, and it also exceeds by a factor of nearly 2 the highest mean for CF#1 mice used in another similar experiment not reported here.

Fig. 1 also illustrates how reactions to the intramuscular test are more intense at twenty-four than at forty-eight hours, an observation that agrees with those of Selbie and O'Grady, and O'Grady.[6, 30] In contrast, lung density and footpad reactions have tended to endure longer, and skin reactions often have been more intense at the forty-eight-hour reading.

TABLE III. PER CENT OF CF#1 MICE REACTING TO THREE TESTS FOR DELAYED HYPERSENSITIVITY*

TEST	CHALLENGE	MOUSE GROUP	
		TEST	CONTROL
Systemic	3.0 mg. H37Ra intravenously	88	0
Temperature	3.0 mg. H37Ra intravenously	100	50
Temperature	0.3 mg. H37Ra intravenously	89	20
Lung density	0.3 mg. H37Ra intravenously	100	30

*Challenges twenty-nine days after first vaccination; readings taken twenty-four hours after challenge except for the forty-eight-hour lung density determination.

The third experiment compares two variations of the systemic tuberculin test with the lung density test in CF#1 mice and presents data on temperature changes in mice undergoing tuberculin shock. Test mice were sensitized

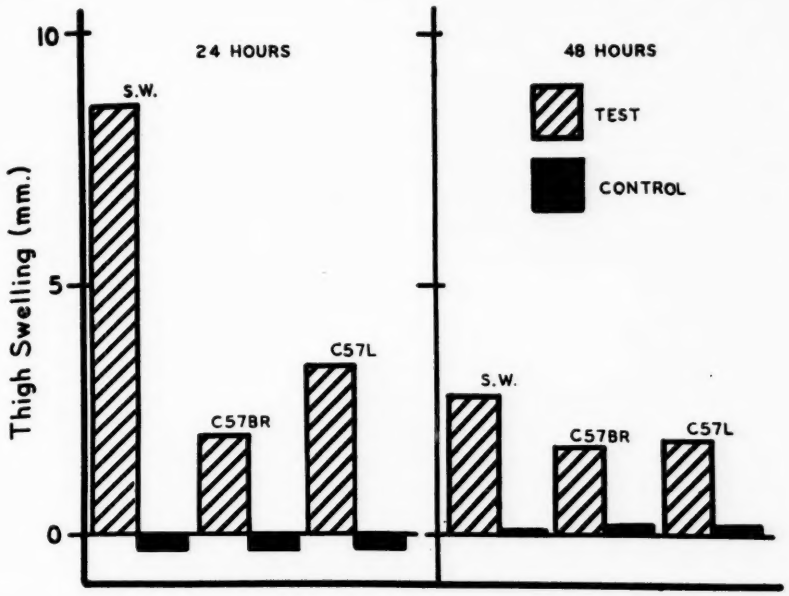

Fig. 1.—Comparison of thigh swelling in three different strains of sensitized (test) and unsensitized (control) mice twenty-four and forty-eight hours after intramuscular challenge with 0.03 mg. of H37Ra tubercle bacilli.

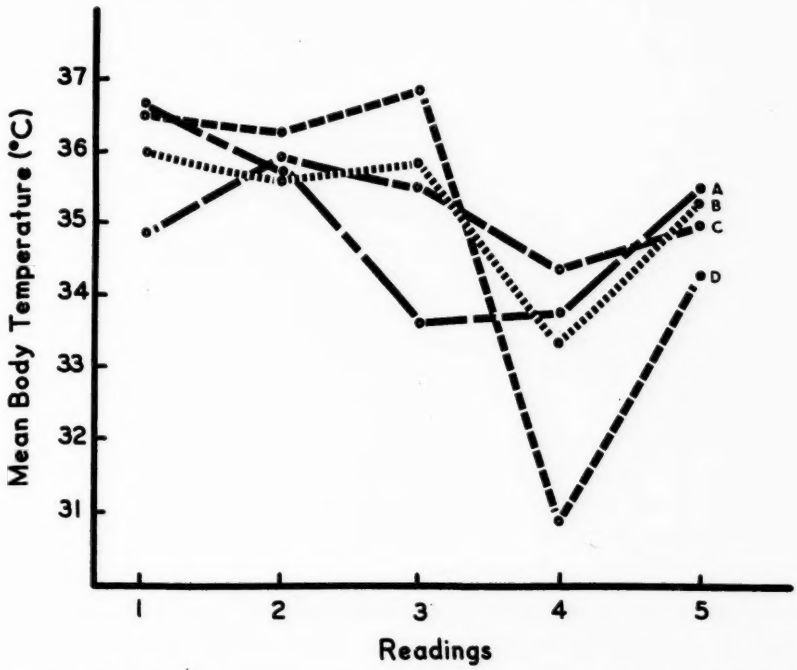

Fig. 2.—Mean body temperatures of sensitized CF#1 mice challenged with 3.0 (*D*) and 0.3 (*B*) mg. of H37Ra bacilli and of unsensitized mice also challenged with 3.0 (*A*) and 0.3 (*C*) mg. of H37Ra bacilli, respectively. Readings were made before vaccination (*1*), just before challenge (*2*), thirty minutes after challenge (*3*), and twenty-four (*4*) and forty-eight (*5*) hours after challenge.

by a single intraperitoneal injection of 1 mg. H37Ra bacilli in w/o emulsion one month before challenge. Control mice received a similar injection but without bacilli. Challenges and their results are presented in Table III, and in Fig. 2 body temperature variations in tuberculin-shocked mice are compared with those in similarly challenged but unsensitized mice.

All three tests differentiated test mice from control mice but not with equal success. Toxicity of the bacillary challenge suspension was higher than anticipated, causing several unsensitized mice to react in two of the tests. However, this very toxicity adds some interesting features to the temperature studies. Fig. 2 shows that it caused significant depression of body temperature in unsensitized mice, with severity depending on the size of the challenge. Superimposed on this drop, apparently due to the primary toxicity of tubercle bacilli, was another severe drop which was most marked twenty-four hours after challenge; this probably was a manifestation of systemic tuberculin shock, since it occurred only in sensitized mice. Although bacillary toxicity had caused a remarkable drop in body temperature in heavily challenged unsensitized mice within thirty minutes, it appeared not to affect sensitized mice. This observation could be interpreted as an immediately occurring antitoxic effect of previous vaccination (immunization), as a portion of the delayed type of reaction, or as an immediate hypersensitivity reaction causing a marked relative body temperature rise.[13]

In view of the generally held opinion that pyrexia is a predominant characteristic of delayed hypersensitivity reactions,[13, 14, 18, 19, 31, 36-38] the significant and specific temperature depressions exhibited in Fig. 2 for sensitized mice are particularly interesting. They resemble rather closely those in sensitized guinea pigs challenged with tuberculoprotein observed by Sabin and colleagues[20] and, more recently, by Friis.[17] One of the sensitized mice that received 3 mg. of tubercle bacilli had become comatose twenty-four hours after challenge and, although still breathing and slightly irritable, had too low a body temperature to register above the 29° C. lower limit of the thermometers being used; it died before the forty-eight-hour reading was made. Other mice in this group, also outwardly suffering severe tuberculin shock, registered temperatures between 30° and 31° C. twenty-four hours after challenge. By the forty-eight-hour reading, however, they were recovering and their temperatures were rising toward normal.

Because bacillary toxicity caused temperatures to drop by a statistically significant 2° C. (probability less than 0.001) in several unsensitized mice, the temperature test as used here was useless in designating individual mice as hypersensitive or not hypersensitive. Nevertheless, the mean temperatures of entire control and test groups were statistically quite different, so that the results in Table III do not show this test's true value as one potentially useful in supplementing others.

Previous reports of systemic tuberculin reactions in mice[2, 6, 7, 23] have been confirmed. Despite the drastic body temperature changes caused in some control mice by bacillary toxicity and the fact, ascertained at autopsy, that

this toxicity provoked lung hemorrhages, none of this group were judged reactive by the observer. Most test mice, on the other hand, showed varying but obvious symptoms of tuberculin shock.

The consistent failures reported in the literature of attempts to elicit skin reactions of the delayed type in mice have been used to buttress arguments that mice develop no tuberculin allergy. However, the first experiment reported in this article suggests that mouse tuberculin skin reactions can be demonstrated under certain conditions. The following experiment presents more proof and illustrates certain characteristics of these reactions.

Several sets of ten test and control mice were vaccinated in the usual fashion. Then various sets were employed in several ways as shown in Table IV.

TABLE IV. PER CENT OF CF#1 MICE REACTING TO FOOTPAD AND INTRACUTANEOUS TESTS UNDER VARIOUS CONDITIONS*

TEST†	CHALLENGE CONDITIONS	MOUSE GROUP	
		TEST	CONTROL
Footpad	First test 21 days after first vaccination	89	0
Intracutaneous	First test 21 days after first vaccination	100	10
Footpad	Second test 3 days after first footpad test	100	0
Footpad	Second test 6 days after first footpad test	80	0
Intracutaneous	Second test 6 days after first footpad test	80	10
Intracutaneous	Third test 3 and 6 days after second and first footpad tests, respectively	90	10
Intracutaneous	Third test 6 days after first footpad and 1 day after systemic‡	90	Not done

*Readings taken forty-eight hours after challenge.
†Footpad, 0.015 mg. H37Ra; intracutaneous, 0.01 ml. of 1 per cent tuberculoprotein.
‡Intraperitoneal injection of 1 mg. tuberculoprotein.

First, primary footpad and skin tests, performed as in previous experiments, were used to measure frequency and degree of sensitization. According to both tests, most or all test mice were sensitive, and nonspecific reactions in the controls were limited. Skin reaction characteristics are detailed below in connection with Table V and Fig. 3.

TABLE V. REACTIONS IN SENSITIZED AND UNSENSITIZED SETS OF CF#1 MICE TWENTY-FOUR AND FORTY-EIGHT HOURS AFTER INTRACUTANEOUS INJECTION OF 0.1 MG. TUBERCULOPROTEIN

MOUSE GROUP	MEAN DIAMETER*	MEAN THICKNESS†
	Twenty-four hours	
Sensitized	3.3	3.0
Unsensitized	1.5	1.8
	Forty-eight hours	
Sensitized	4.6	3.6
Unsensitized	1.1	1.8

*In millimeters.
†Thickness of reaction center relative to surrounding normal skin thickness.

Mice and other animals with the immediate type of hypersensitivity are desensitized by a single injection of eliciting antigen sufficient to cause anaphylaxis and remain so for several days.[1, 13, 24, 25] Delayed hypersensitivity is not so easily abolished, a lengthy course of antigen injections being required.[14, 26, 27, 40] If repeated footpad tests and skin tests, or a skin test done

after anaphylactic desensitization, were to yield reactions of practically undiminished frequency and intensity, then both tests would be somewhat more firmly established as specific for delayed hypersensitivity. Such was the case, according to results in Table IV. Footpad and intracutaneous secondary challenges on mice tested a few days before elicited reactions as readily as could be expected in sensitized but previously untested mice.

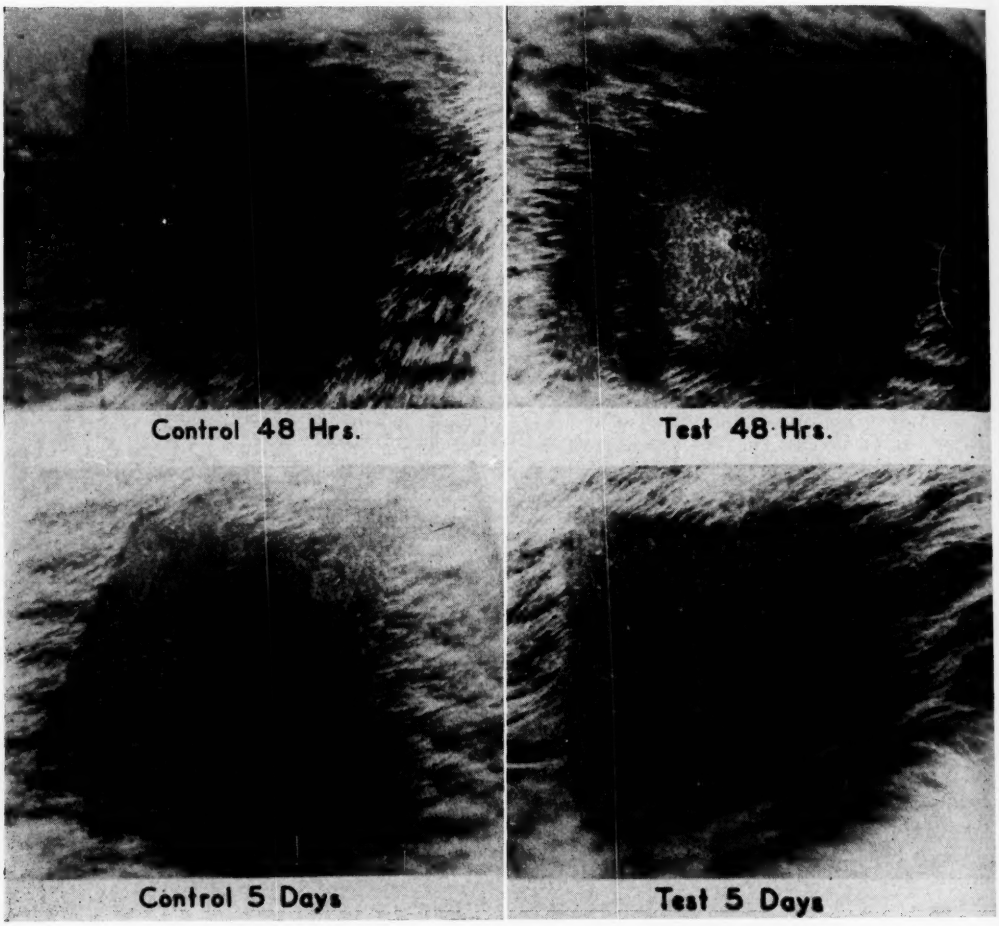

Fig. 3.—Reactions of sensitized (test) and unsensitized (control) CF#1 mice two and five days after challenge with 0.01 ml. of 1 per cent tuberculoprotein injected intracutaneously.

Intracutaneous tests also were successful in sensitized mice first footpad-tested and then, twenty-four hours before skin-testing, injected with sufficient tuberculoprotein to desensitize anaphylactically. This desensitizing intraperitoneal tuberculoprotein injection caused moderate anaphylaxis followed within an hour by complete recovery and, in turn, succeeded the next day by moderate systemic tuberculin reaction. Thus, it appears that anaphylactically desensitized mice undergoing moderate tuberculin shock at the time they

were skin-tested for tuberculin allergy still were capable of reacting in the delayed fashion. However, results not shown in Table IV suggest that these reactions may have been somewhat less intense than in mice in which desensitization had not been attempted.

Fig. 3 compares the appearance of tuberculin skin reactions in sensitized mice two and five days after challenge with similarly tested skin sites on unsensitized mice, and Table V gives numerical data for the reactions. Except for their lack of erythema, these skin reactions resemble those seen in other animals. A typical reaction developed as follows: Intracutaneous injection formed a bleb which usually disappeared during the next hour, leaving a low mound of blanched skin. Neither edema nor petechiae, sometimes seen shortly after challenge in mice with strong anaphylactic hypersensitivity,[28] was definitely seen, but usually after six hours had passed (and invariably by the twenty-four-hour reading) induration exceeding that occasionally appearing nonspecifically could be distinguished and its diameter and relative thickness measured. Induration diameter increased during the next twenty-four-hour period and then very slowly diminished, commonly being obvious as much as a week after challenge. As it diminished it tended to leave wrinkled skin with low elasticity, and in severe reactions (but not with a predictability which would prompt understanding) a central, shallow skin necrosis appeared by the forty-eighth hour and tended to spread.

DISCUSSION

These data may help diminish misunderstanding of delayed hypersensitivity in the mouse. A comparison of sensitization in several mouse strains and methods for detecting it shows that in many respects it resembles that seen in other animal species and in man.

Despite their complexity, tubercle bacilli and their protein were chosen as sensitizing and eliciting antigens for several reasons. The experimental background is greater for tuberculin allergy than for any other delayed type of allergy. Tuberculin allergy is known to be important in tuberculosis pathogenesis, but the interrelationship is poorly understood because of lack of information of the kind contributed here. In preliminary experiments with other antigens, sensitization would most logically be attempted with the aid of Freund's adjuvant (a w/o emulsion of killed mycobacteria) as the surest approach. It would seem simpler to use these bacilli themselves as the sensitizing antigen. The traditional method of sensitizing mice by infecting them with virulent tubercle bacilli was avoided as impractical and poorly controllable.

Mouse strain differences could be imputed to account for frequent disagreement among various laboratories which have shown experimental interest in mouse tuberculin allergy.[1] This view is not upheld by our experiments, however, for they have shown that all but one of seven inbred strains develop delayed hypersensitivity with nearly equal frequency. The exceptional strain, C3H, seems to be less frequently sensitized, but to our knowledge it has never

been reported used in tuberculin allergy studies. Interestingly, however, Fink and Rothlauf[39] report that, of several strains of mice used in studies of anaphylaxis, C3H mice were significantly the least often reactive. Another strain, Swiss Webster, distinguishes itself not in frequency of sensitization but rather in reacting more strongly, once it is sensitized, than mice of the other six strains, as measured by the intramuscular test. Since its stronger reactions are suggested but not entirely substantiated by concomitantly done footpad and systemic tests, we are not certain whether it has greater hypersensitivity or merely reacts more strongly to intramuscular challenge.

C57BL mice sensitized to H37Ra (human variety) tubercle bacilli must be noted as having cross-reacted more poorly than three other strains to intramuscular challenge with bovine variety (BCG) bacilli. Cross-reactions of this kind in other animals and man often are weaker than homologous reactions,[22, 29] but perhaps this strain of mice is particularly discriminative.

All six techniques used to measure delayed hypersensitivity reactions were functional, but not all were equally useful. According to sensitivity, convenience, and reliability, they can be divided into primary and secondary tests. Primary tests include the footpad, intracutaneous, and intramuscular tests, whereas lung density, body temperature, and systemic tests are secondary.

The value of the footpad test in C57BL mice has been attested by its usefulness to Gray and co-workers,[3, 4] but it did not succeed in the hands of Gastinel and associates,[2] who used white mice. The present experiments confirm its utility in the black mice and show that it can be applied equally well in other mouse strains, including albinos. Its virtues are easy performance, simple reading, and infrequency of nonspecific reaction complications. Its sensitivity appears to at least equal that of any other test employed, and it has proved reliable. Disadvantages include subjectivity of reading, which limits its quantitative and comparative usefulness, and the physical impracticality of performing several or very frequently repeated tests on a single mouse.

The intracutaneous test proved more useful for detecting delayed hypersensitivity in the mouse than previous published experiments with it[2, 5, 21, 30] led us to expect. As presently used and as compared with the footpad test, it is not so reliable, it seems to be relatively less sensitive, and it requires more skill to perform properly. Its reliability is most limited by the fairly high incidence of nonspecific reactions in unsensitized mice. In terms of a ratio of challenge protein to tissue obviously involved in their respective reactions, the footpad's reaction to 0.015 mg., wet weight, of tubercle bacilli (approximately equivalent to 0.003 mg. of dry bacilli or 0.0006 mg. of somatic tuberculoprotein[31]) has the appearance of being more intense than the intradermal reaction to 0.1 mg. of culture filtrate tuberculoprotein. Had entire bacilli or somatic protein been used in the skin test, however, more intense specific reactions might have been elicited by a possibly higher challenge antigen reactivity, so that the point of relative test sensitivity remains

speculative. Advantages of the skin test are its objectivity and its greater adaptability to such operations as multiple simultaneous tests on one mouse, local passive transfer experiments, and detection of simultaneously occurring immediate and delayed types of hypersensitivity.

Selbie and O'Grady[6] detected tuberculin allergy in white mice by injecting them intramuscularly in the thigh and then recording increased thigh diameter as a measure of reaction. Their test, with the reading method slightly modified, has been used here equally well in pigmented and white mice and has been found easy, sensitive, objective, and reliable. It is the easiest of the primary tests to perform, but reading its reactions accurately can be somewhat tedious. Even more than the footpad test, it is of limited use in simultaneous multiple-test experiments. Recovery from it is faster, however, so that performing several successive tests on one animal presents little problem,[30] providing test antigen toxicity is low and the antigen is not particulate.

The merits of the lung density test have been presented elsewhere.[1] Although objective, quantitative, and sensitive, it cannot rank as a primary test because technically it is difficult and its reliability is encumbered by an undesirably high incidence of nonspecific or toxic reactions among unsensitized mice. It can be used only once in a given mouse. On the other hand, it is the only test among the six used which measures delayed hypersensitivity reactions in the major organ, the lungs, whose progressive functional destruction by tubercle bacilli may very likely be strongly influenced by these reactions in mice suffering from chronic tuberculosis, in view of the pathologic and bacteriologic findings of Sever and Youmans.[32] Probably, then, it will see use as a specialized test for any kind of toxicity, primary or secondary, which affects lungs, and it shows promise of playing an important role in improving our understanding of the pathogenesis of tuberculosis.

Body temperature and systemic tests can be considered jointly. The latter is so insensitive as to be interesting relative to our experiments only in confirming its previous successful use in mice.[2, 6, 7, 23] It is easy to perform but not easy to read. By contrast, the body temperature test, although only moderately reliable among individual mice in its unrefined use described here, may turn out to be useful frequently in future experiments on mouse delayed hypersensitivity, systemic toxicity reactions, and related problems. Readings are made easily and entirely objectively, and when handled in groups they are highly reliable statistically. Their interpretation is likely to be complicated by frequent toxic reactions in unsensitized mice, but these reactions themselves are interesting. In short, if it is used under carefully controlled conditions, the body temperature test, like the lung density test, has virtues which make it potentially particularly useful in certain kinds of experiments.

Body temperature changes have been employed in man and in various animal species as both primary and secondary indicators of systemic tuberculin reactions.[13-17, 20, 33-36] They have been used for detecting tuberculosis

in cattle[33]; a cow was considered reactive if it developed a fever of 2° F. or more between the eighth and eighteenth hours after challenge. Tuberculin reactions in human beings are accompanied by high fevers.[14, 19, 36-38] Students of tuberculin allergy in various species of laboratory animals (guinea pigs, rabbits, sheep) have reported that temperatures rise in these animals a few hours after challenge, remain high for a while, and then return to normal in eight to twelve hours.[34, 35] Such data as these have fostered the mistaken notion that temperature elevation is a distinguishing characteristic of delayed hypersensitivity reactions.[13] Actually, temperature variations during tuberculin shock seem to depend entirely upon how severe the relative challenge is. Mild challenge induces temporary fever which disappears without complications. Severe challenge, however, can lead directly to a precipitous temperature drop followed by coma and death, or it can evoke a short-lived fever and subsequent prolonged temperature depression.[16, 17, 20] Whether there will be fever, fever followed by temperature drop, or only the temperature drop appears to be governed by the balance between strength of challenge and degree of hypersensitivity.

In the experiments reported here, the sensitized mice appear to have developed both temporary fever and subsequent fall in temperature much as has been reported for sensitized guinea pigs.[17] A temporary rise above normal body temperature was not seen, but pyrexia in test mice is suggested by the fact that bacillary toxicity caused significant temperature depression among control mice (see Sabin and associates[20] for similar observations in guinea pigs) but not in test mice thirty minutes after challenge. This pyrexia conceivably might not be a part of the delayed hypersensitivity syndrome but, rather, a manifestation of simultaneously provoked mild anaphylactic shock. From another point of view, however, relative fever may have been absent, test mice simply having acquired some kind of protection against bacillary toxicity through vaccination so that they were not affected as control mice were.

In mice, tuberculin shock appears to be the most severe twenty-four hours after challenge. Similarly, both here and in O'Grady's[6, 30] laboratory, reactions to the intramuscular test were found to peak one day after challenge. On the other hand, footpad and skin reactions are longer lasting, and although the former have tended to be less severe at the forty-eight-hour reading than at the twenty-four-hour reading, skin reactions often have been more severe and not uncommonly have lasted for as much as a week even though a soluble antigen was utilized. These findings lead to a reasonable supposition that reactions of the delayed type depend partly in their timing on the rapidity with which reactive body elements confront the antigen and on the susceptibility of the antigen, either by its nature or by its route of injection, to removal from the injection site, a point also suggested in part by O'Grady[30] when he found Old Tuberculin given intramuscularly to "desensitized" mice unable to elicit a reaction when one still could be evoked by entire tubercle bacilli. This subject might be investigated profitably in some detail with regard to allergic diseases and to infectious diseases, such as

tuberculosis, in which allergy may play a major role. For example, to elicit lung density increases in sensitized mice, tubercle bacilli are best injected intravenously; yet, as shown in a previous report,[1] the lung density reaction is the most intense between two and four days after challenge, a finding which might not be expected from generalizations drawn from the systemic and body temperature tests, unless for some reason either the antigen (tubercle bacilli) is not eliminated from lungs as rapidly as it is from other body organs or the delayed hypersensitivity response of lungs is slower and more prolonged than that of most other body tissues. This might explain, in part, why mouse tuberculosis is pulmonary.

Numerous criteria are available for differentiating between immediate and delayed types of hypersensitivity. In order not to go beyond the scope of this article or to slight a subject worthy itself of careful exposition, I have presented only two criteria here as evidence that skin reactions of the delayed type were elicited specifically in sensitized mice.

The first is descriptive. Skin reactions of the immediate type are edematous and accompanied by intracutaneous bleeding; induration and central necrosis are characteristic of the delayed type of reaction.[13, 14, 28] When both types of hypersensitivity occur together, all four signs may be seen,[28] but in the present experiments only induration and central necrosis were observed. Erythema usually accompanies both types of reaction in the larger animals and in man, but it was not noticed in these mice.

The second criterion rests on the relative ease with which sensitized animals can be desensitized. A single large injection of antigen temporarily abolishes immediate hypersensitivity,[1, 13, 24, 25] but delayed hypersensitivity can be weakened significantly only by a long course of repeated antigen injections.[14, 26, 27, 37, 40] Repeated tests did not affect mouse reactivity within the limits of our present experiments, and injection of a quantity of tuberculoprotein known from prior experience to desensitize to anaphylactic hypersensitivity[1] did not diminish the delayed skin reactivity. Together, this and the descriptive data above offer preliminary evidence that these skin reactions truly are tuberculin reactions.

SUMMARY

Delayed hypersensitivity (tuberculin allergy) was induced in seven different strains of mice and detected by means of six different kinds of tests in order to compare both the tests and mouse strains under standardized conditions. The mice were sensitized by two weekly subcutaneous injections of living H37Ra tubercle bacilli suspended in water-in-oil emulsions, and they were tested three weeks after the first vaccination.

C57BL, C57BR, C57L, DBA, and CF#1 mouse strains were not essentially different, either in frequency or in intensity of hypersensitization. C3H mice appeared to be less frequently sensitized than mice of other strains but, when sensitive, reacted as strongly. Swiss Webster mice were sensitized as frequently but reacted somewhat more intensely.

The six tests employed were divided into primary and secondary categories by reliability, sensitivity, versatility, and simplicity. Footpad, intracutaneous, and intramuscular tests made up the first category; lung density, body temperature, and systemic tests comprised the second.

Some basic data concerning delayed hypersensitivity in the mouse as well as its detectability by the traditional skin test have been presented and discussed.

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BLOOD GROUPS AND SECRETION OF BLOOD GROUP SUBSTANCES

Comparison of Allergic With Nonallergic Persons in a Pacific Northwest College Population

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With the technical assistance of Gabrielle Bouchard and Sue Stevens

SEVERAL associations have emerged from the many studies concerning the distribution of human blood groups in disease. The predominance of group O in patients with peptic ulcer has been repeatedly confirmed, and the association of group A with gastric carcinoma is also well established. These associations have been well discussed by Fraser Roberts[1] in a recent critical review of blood groups and disease. Another remarkable correlation is that reported to occur between group A and salivary tumors.[2] The significance of such relationships is as yet obscure, but their occurrence has stimulated an extensive search for other disease-blood group correlations.

Many studies of blood groups in disease can be criticized because of their dependence on small numbers and on control data from separate populations (such as blood donors). The several defects in blood group studies were well outlined by Fraser Roberts[1] and re-emphasized by Manuila.[3] These pitfalls need to be considered seriously in outlining any new study of blood group associations. Special attention was therefore given to the establishment of adequate controls in the present study of blood group distribution in persons with asthma and hay fever.

The tendency to develop these allergic disorders is heritable, and their occurrence in an unselected population is common enough to permit a large number of persons to be evaluated. These features allowed us to determine the blood group distribution and secretor status in a relatively homogeneous college student population containing both the "normal" and "allergic" groups.

MATERIALS AND METHODS

All students who registered for the first time at the University of Washington in Seattle between December, 1956, and October, 1957, answered a detailed questionnaire concerning the presence or absence of a history of asthma or hay fever. Twelve per cent reported hay fever only, 2.7 per cent reported asthma and hay fever, and 2.0 per cent reported asthma alone; a total of 16.7

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Assisted by grants from Ciba Pharmaceutical Products, Inc., the Washington State Heart Association, and the National Institutes of Health.

Received for publication Feb. 26, 1959.

per cent indicated a history of one or both of these allergic disorders. Further details will be reported elsewhere.[4] Negro and Oriental students, who would be expected to show somewhat different blood group frequencies, were excluded.

Blood was obtained by venipuncture from all subjects and allowed to clot. Within six hours of collection, we performed ABO grouping on the blood specimens, using high-potency antiserum.* From July to October, 1957 (representing the largest registration group), almost all samples (4,387) were tested in addition with anti-D serum,* and 1,125 of 1,922 group A bloods were separated into A_1 and A_2 subgroups by a combined approach involving absorbed anti-A serum and plant lectins which has been reported previously.[5]

Finally, starting in July, 1957, salivas were collected from all students with positive allergic histories and from 2,355 nonallergic students. A total of 3,144 salivas were tested for presence of blood group substance by the use of an extract of *Ulex europaeus* seeds in a hemagglutination inhibition technique described by Boyd and Shapleigh[6] and modified for large-scale use.[5]

RESULTS AND DISCUSSION

The ABO grouping of 5,657 persons is presented in Table I. The over-all incidence of group O was 42.4 per cent and of group A, 43.4 per cent. There was no significant difference between the allergic and nonallergic groups ($\chi^2 = 0.77$, $p > 0.3$). The reason for the difference in A and O blood group frequencies between male and female subjects in the nonallergic group ($\chi^2 = 6.7$, $p = 0.01$) is not clear. The difference was not found within the allergic group.

TABLE I. INCIDENCE OF BLOOD GROUPS IN ALLERGIC AND NONALLERGIC STUDENTS

	O		A		B		AB		
	NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT	NUMBER	PER CENT	TOTAL
Allergic									
Males	261	41.9	277	44.5	59	9.5	26	4.2	623
Females	122	40.3	139	45.9	36	11.9	6	2.0	303
Total	383	41.4	416	44.9	95	10.3	32	3.5	926
Nonallergic									
Males	1,484	43.8	1,427	42.1	361	10.6	120	3.5	3,392
Females	532	39.7	615	45.9	143	10.7	49	3.7	1,339
Total	2,016	42.5	2,042	43.3	504	10.6	169	3.6	4,731
Total group									
Males	1,745	43.4	1,704	42.4	420	10.4	146	3.6	4,015
Females	654	39.8	754	45.9	179	10.9	55	3.3	1,642
Grand total	2,399	42.4	2,458	43.4	599	10.6	201	3.6	5,657

The slight excess of blood group A_2 in the allergic population (Table II) is not significant ($\chi^2 = 1.6$, $p > 0.2$). Also, typing with anti-D serum did not reveal any significant abnormalities in the allergic group (Table III). However, the fraction of d (Rh-negative) was higher in female subjects of both allergic and nonallergic groups than in males; this difference was statistically significant ($\chi^2 = 6.7$, $p = 0.01$).

*Obtained from the Ortho Pharmaceutical Corporation, Raritan, New Jersey.

TABLE II. A_1-A_2 DISTRIBUTION, ALLERGIC VERSUS NONALLERGIC STUDENTS

	A_1	A_2	A_1B	A_2B	TOTAL	PER CENT A_1 OR A_1B
Allergic						
Males	143	43	10	6	202	75.7
Females	71	19	2	2	94	77.7
Total	214	62	12	8	296	76.4
Nonallergic						
Males	418	111	36	12	577	78.7
Females	187	37	21	7	252	82.5
Total	605	148	57	19	829	79.9
Total group						
Males	561	154	46	18	779	77.9
Females	258	56	23	9	346	81.3
Grand total	819	210	69	27	1,125	79.0

TABLE III. DISTRIBUTION OF D AND d BLOOD TYPES AMONG ALLERGIC AND NONALLERGIC GROUPS

	O			A			B			AB			TOTAL		
	D	d	% D	D	d	% D	D	d	% D	D	d	% D	D	d	% D
Allergic															
Males	165	38	81.3	175	30	85.4	34	10	77.3	15	5	75.0	389	83	82.4
Females	85	18	82.5	83	25	76.8	25	4	86.2	5	0	100.0	198	47	80.8
Both	250	56	81.7	258	55	82.4	59	14	80.8	20	5	80.0	587	130	81.9
Nonallergic															
Males	971	183	84.0	902	172	83.9	249	60	80.6	89	11	89.0	2,211	426	83.9
Females	309	94	76.8	383	88	81.3	105	15	87.5	32	7	82.0	829	204	80.1
Both	1,280	277	82.3	1,285	260	83.2	354	75	82.5	121	18	87.1	3,040	630	82.8

Distribution of the above-mentioned factors was also calculated separately for the 212 persons within the allergic population with a history of asthma. Since the results were practically identical with the data already presented for the total population, they were not tabulated separately.

The number and percentage of secretors of H substance is presented in Table IV, according to blood groups and allergic history. There are fewer nonsecretors among the allergic persons than among the remainder (21.5 per

TABLE IV. SECRETOR STATUS OF ALLERGIC AND NONALLERGIC GROUPS

	O		A		B		AB		TOTAL			
	S	s	S	s	S	s	S	s	S	s	BOTH	% s
Allergic												
Males	166	39	186	45	31	20	16	5	399	109	508	21.4
Females	89	30	99	25	28	5	4	1	220	61	281	21.7
Both	255	69	285	70	59	25	20	6	619	170	789	21.5
Nonallergic												
Males	523	162	520	168	136	45	39	11	1,218	386	1,604	24.4
Females	255	83	231	85	44	28	20	5	550	201	751	26.8
Both	778	245	751	253	180	73	59	16	1,768	587	2,355	24.9
Total												
Males	689	201	706	213	167	65	55	16	1,617	495	2,112	23.4
Females	344	113	330	110	72	33	24	6	770	262	1,032	25.4
Both	1,033	314	1,036	323	239	98	79	22	2,387	757	3,144	24.1

S = Secretor.
s = Nonsecretor.

cent versus 24.9 per cent, $\chi^2 = 3.71$, $p = 0.055$). In the 181 asthmatic persons tested, the incidence was identical (Table V). A similar trend was first noted by McConnell and Sheppard[7] in 250 persons with active asthma, but the

TABLE V. SECRETOR STATUS OF ASTHMATIC GROUP

	S	s	TOTAL	% s
Males	97	29	126	23.0
Females	45	10	55	18.2
Both	142	39	181	21.5

S = Secretor.
s = Nonsecretor.

variability in their data (particularly between males and females) rendered statistical evaluation difficult. In our study, the level of significance of this association, although not at all conclusive, suggests that further studies might be worth while.

SUMMARY

The blood group distribution of 5,657 mixed-white college students from the Pacific Northwest was found to be 42.4 per cent O, 43.4 per cent A, 10.6 per cent B, and 3.6 per cent AB. Seventy-nine per cent of 1,125 group A persons were A_1. Eighty-three per cent of 4,387 students were Rh-positive, and 24.1 per cent of 3,144 were nonsecretors of blood group substance.

There were no significant differences in such distributions within this population between allergic (hay fever and asthma) and nonallergic persons.

The cooperation of Gordon G. Bergy, M.D., Director of the Hall Health Center, University of Washington, and his staff is gratefully acknowledged.

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PARALLEL VOLUMETRIC AND GRAVIMETRIC POLLEN COUNTS

The Variabilities and Inconsistencies of a Conversion Factor

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POLLEN counts are now being made by allergists throughout the nation as a method of estimating the severity of a particular hay fever season. Pollen counting methods have been under constant investigation in an effort to make pollen counting a more exact and scientific operation. At present, most pollen counts are done by the gravity slide method using the standard pollen shelter and then converting the slide area count into a volumetric equivalent by the use of an appropriate conversion factor.* In adopting the standard procedure in 1947, the National Pollen Survey Committee of the American Academy of Allergy,[1] reported, in part: "There is no simple volumetric device now available for directly measuring the pollen content of the air which could be readily applied on a nation-wide scale. Under the circumstances, the gravity method, although subject to many limitations, provides the only practical counting procedure which can be universally employed and must therefore be maintained. For the present, a conversion factor based upon actual comparative determinations made with a standard gravity sampling technique and a reliable volumeteric sampler represents the only practical approach to the solution of the problem."

The first attempts to convert surface area counts to their volumetric equivalent was the 1.8 factor that Scheppegrel[2] adopted for ragweed pollen in 1922. This was calculated through the use of Stokes's law, which depends upon the rate of fall of small, smooth bodies in still air. Cocke,[3, 4] in 1937, suggested that errors existed in Scheppegrel's original calculations and later ran some parallel volumetric and gravimetric determinations to evaluate the tables. Dahl and Ellis,[5] in 1942, further disapproved these figures and recommended that they be re-evaluated. Durham,[6-10] recognizing the inadequacies of this method, made numerous parallel gravimetric and volumetric determinations between 1942 and 1945 in an effort to derive a satisfactory conversion factor. As a result of seventeen tests covering 136 hours of parallel observations, he obtained a figure of 3.6 for ragweed pollen. This factor has been used by allergists and aerobiologists since 1947.

From the Albert Einstein Medical Center.

Read before the fifteenth annual meeting of the American Academy of Allergy, Chicago, Illinois, Feb. 9, 1959.

Received for publication Feb. 26, 1959.

*The terms "volumetric" and "gravimetric" are used in this article to denote an aerobiologic method of air sampling, and not in the standard chemical sense.

In an effort to standardize our methods further, it seemed worth while to validate the accuracy of the currently used conversion factor. The present communication deals with the results of numerous parallel gravimetric and volumetric observations and calculations regarding a conversion factor. The volumetric apparatus used in these experiments has been reported on previously[11] and fulfills all the requirements for a standard sampling device. Previous experiments[12] show that when identical apparatuses were used at variable

TABLE I. PARALLEL VOLUMETRIC AND GRAVIMETRIC COUNTS: EFFECT OF WIND ORIENTATION

DATE	NONORIENTED	WIND-ORIENTED
8/28	1,798	2,378
8/29	2,465	2,561
9/2	52,811	51,901
9/4	20,693	21,836
9/8	5,627	5,525
9/9	1,338	1,381
9/10	1,971	1,900
9/11	1,578	1,397
9/12	2,457	2,369

Figures represent total counts for twenty-four hours at a flow rate of 10 liters per minute.

speeds, the results were almost the same after corrections were made for total volumes drawn. During the 1958 ragweed season, a wind-oriented apparatus was devised and compared to our standard sampler which was drawing the same volume of air. Orientation of the intake spout to wind direction had no appreciable effect on the total pollen catch, as shown by the daily and hourly counts for both apparatuses in Tables I and II.

TABLE II. PARALLEL VOLUMETRIC AND GRAVIMETRIC COUNTS: EFFECT OF WIND ORIENTATION SEPT. 4, 1958

HOUR	NONORIENTED	WIND-ORIENTED
M	148	143
1	180	148
2	191	186
3	244	254
4	285	270
5	371	355
6	647	663
7	1,145	2,046
8	1,707	1,447
9	1,389	1,590
10	922	991
11	933	853
12	530	447
13	774	1,056
14	806	769
15	647	641
16	859	999
17	1,134	1,118
18	1,113	1,113
19	1,187	1,002
20	1,177	1,590
21	1,304	1,299
22	1,611	1,542
23	1,389	1,394
	20,693	21,836

Figures represent hourly counts at a flow rate of 10 liters per minute.

During the 1957 ragweed season, two Marx volumetric air-sampling devices and one standard gravity device were exposed, side by side. All apparatuses were operated for a total of forty-six days, or 1,104 hours each. The daily volumes were reduced to their cubic yard equivalents, and the gravity slide counts were reduced to square centimeter values. A comparison was then made between the volumetric and gravimetric figures for each day. This we

TABLE III. PARALLEL VOLUMETRIC AND GRAVIMETRIC COUNTS, 1957
VOLUMETRIC IMPINGER NO. 1, 10 LITERS PER MINUTE

DATE	GRAV./CM.^{2}	VOL./YD.^{3}	VOLUMETRIC-GRAVIMETRIC RATIO
Aug. 15	4	3	0.7
16	3	20	6.6
17	4	14	3.5
18	3	25	8.3
19	5	35	7.0
20	24	70	2.8
21	12	46	3.8
22	10	43	4.3
23	11	83	7.5
24	6	87	14.5
25	6	28	4.6
26	4	29	7.2
27	17	77	4.5
28	15	41	2.7
29	13	90	6.9
30	26	129	4.9
31	30	342	9.5
Sept. 1	63	560	8.8
2	56	637	11.3
3	62	616	9.9
4	48	580	12.1
5	91	1,000	10.9
6	29	614	21.1
7	31	483	15.6
8	26	262	10.0
9	22	122	5.5
10	6	521	8.6
11	10	139	13.9
12	14	159	11.3
13	38	149	3.9
14	43	366	8.5
15	25	156	6.2
16	9	95	10.5
17	16	140	8.7
18	17	155	9.1
19	10	209	20.9
20	17	116	6.8
21	8	80	10.8
22	11	40	3.6
23	4	38	9.5
24	3	25	8.3
25	4	37	9.2
26	9	58	6.4
27	3	50	16.3
28	2	6	3.0
29	1	25	25.0

Mean volumetric-gravimetric ratio = 8.8
Standard deviation = 4.8
Coefficient of variation* = 55%

*Coefficient of variation = $\frac{\text{Standard deviation}}{\text{Mean}}$.

designated the daily volumetric-gravimetric ratio. From these daily ratios, a mean volumetric-gravimetric ratio was then calculated for each piece of apparatus. A high degree of variability in the daily ratios on both pieces of apparatus was noted. These ranged from 0.7 to 25.0, with a mean of 8.8, on one

TABLE IV. PARALLEL VOLUMETRIC AND GRAVIMETRIC COUNTS, 1957
VOLUMETRIC IMPINGER NO. 2, 5 LITERS PER MINUTE

DATE	GRAV./CM.^{2}	VOL./YD.^{3}	VOLUMETRIC-GRAVIMETRIC RATIO
Aug. 15	4	6	1.5
16	3	11	3.6
17	4	12	3.0
18	3	21	7.0
19	5	19	3.8
20	24	63	2.6
21	12	42	3.5
22	10	46	4.6
23	11	88	8.0
24	6	51	8.5
25	6	26	4.6
26	4	26	6.5
27	17	28	1.6
28	15	34	2.2
29	13	91	7.0
30	26	158	6.1
31	30	361	10.0
Sept. 1	63	650	10.3
2	56	668	12.1
3	62	635	10.1
4	48	514	10.7
5	91	975	10.7
6	29	575	19.8
7	31	476	15.3
8	26	272	10.4
9	22	132	6.0
10	6	59	9.6
11	10	136	13.6
12	14	171	12.2
13	38	142	3.7
14	43	371	8.6
15	25	145	5.8
16	9	94	10.4
17	16	133	8.3
18	17	153	9.0
19	10	202	20.2
20	17	110	6.4
21	8	73	9.1
22	11	38	3.4
23	4	41	10.2
24	3	31	10.3
25	4	44	11.0
26	9	57	6.3
27	3	49	16.3
28	2	0	0.0
29	1	27	27.0

Mean volumetric-gravimetric ratio = 8.5
Standard deviation = 4.7
Coefficient of variation = 55%

impinger (Table III) and from 0 to 27.0, with a mean of 8.5, on the second impinger (Table IV). Since the range of ratios appeared to be so broad and since the means of 8.8 and 8.5 were at variance with Durham's factor of 3.6,

the entire set of figures was subjected to statistical analysis. As a result of this analysis, a coefficent of variation of 55 per cent was obtained in each case. For biologic data of this type, a coefficient of variation of 5 to 15 per cent is considered significant. The high coefficient of variation obtained in our series pointed to the unreliability of the volumetric-gravimetric ratios for converting surface areas to their volumetric equivalents.

TABLE V. PARALLEL VOLUMETRIC AND GRAVIMETRIC COUNTS, 1958
VOLUMETRIC IMPINGER NO. 1, 10 LITERS PER MINUTE

DATE	GRAV./CM.^{2}	VOL./YD.^{3}	VOLUMETRIC-GRAVIMETRIC RATIO
Aug. 21	6	70	11.7
22	21	30	1.4
23	10	61	6.1
24	4	6	1.5
26	2	94	47.0
27	23	295	12.8
28	25	96	3.8
29	14	131	9.4
30	38	457	12.0
31	47	898	19.1
Sept. 1	56	1,148	20.5
2	106	2,809	26.5
3	67	1,273	19.0
4	42	1,101	26.2
5	62	1,838	29.6
6	32	1,064	33.2
7	35	245	7.0
8	109	299	10.0
9	25	71	2.8
10	18	105	5.8
11	5	84	16.8
12	6	131	21.8
13	14	361	25.8
14	20	621	31.0
15	29	231	8.0
18	5	111	22.2
19	11	147	13.4
20	7	282	40.3
22	4	111	27.8
23	3	94	31.3
24	8	180	22.2
25	2	9	4.5
27	4	86	21.5

Mean volumetric-gravimetric ratio = 17.9
Standard deviation = 11.1
Coefficient of variation = 61.4%

To test this supposition further, the study was repeated in 1958. One apparatus yielded thirty-two daily calculations over a period of 768 hours, and another gave thirteen daily calculations with 312 hours of observation. In this series, both volumetric devices drew 10 liters of air per minute. The daily volumetric-gravimetric ratios ranged from 1.4 to 47.0, with a mean of 17.9, in apparatus No. 1 (Table V) and from 2.9 to 39.7, with a mean of 16.2, in apparatus No. 2 (Table VI). On analysis, coefficients of variation were 61 per cent and 83 per cent, respectively. The high coefficients of variation and the diverse volumetric-gravimetric ratios again seemed to indicate that these figures had no statistical validity.

TABLE VI. PARALLEL VOLUMETRIC AND GRAVIMETRIC COUNTS, 1958
VOLUMETRIC IMPRINGER NO. 2, 10 LITERS PER MINUTE

DATE	GRAV./CM.^{2}	VOL./YD.^{3}	VOLUMETRIC-GRAVIMETRIC RATIO
Aug. 28	91	126	5.0
29	53	136	9.7
31	170	930	19.8
Sept. 2	384	2,760	26.0
4	152	1,161	27.6
8	109	293	9.8
9	90	73	2.9
10	64	101	5.6
11	17	74	14.8
12	21	126	21.0
15	103	236	8.1
18	18	105	21.0
20	25	278	39.7

Mean volumetric-gravimetric ratio = 16.2
Standard deviation = 13.4
Coefficient of variation = 82.7%

It seemed peculiar that figures that agreed so closely for a single year (8.8 and 8.5) could be so divergent in subsequent years (16.2 and 17.9). In searching for an answer, we observed that Durham's figures in similar experiments also varied considerably (Table VII). Thus, in 1942, his calculations from thirty-two tests involving 285 hours yielded a volumetric-gravimetric

TABLE VII. PARALLEL VOLUMETRIC AND GRAVIMETRIC COUNTS

INVESTIGATOR	METHOD	DURATION OF STUDY	VOLUMES SAMPLED	CONVERSION FACTOR	STANDARD DEVIATION	COEFFICIENT OF VARIATION (PER CENT)
Scheppegrel (1917)	Stokes's law (sp. gr. and rate of fall)			1.8		
Durham (1942)	Parallel volumetric and gravity devices	32 tests 285 hours	0.1 cu. ft./min.	5.39	3.8	70
(1943)		8 tests 52 hours	0.1 cu. ft./min.	6.39	9.8	153
(1943)		8 tests 52 hours	1.0 cu. ft./min.	6.71	6.1	91
(1946)		17 tests 136 hours	0.1 cu. ft./min.	3.6	2.0	55
Blumstein and Spiegelman (1957)		46 tests 1,104 hours	10 L./min.	8.8	4.8	55
(1957)		46 tests 1,104 hours	5 L./min.	8.5	4.7	55
(1958)		32 tests 792 hours	10 L./min.	17.9	11.1	61
(1958)		13 tests 312 hours	10 L./min.	16.2	13.4	83

ratio of 5.39. In 1943, factors of 6.39 and 6.71 were derived from two different sets of observations. When subjected to statistical analysis, the coefficient of variation for the 1942 calculation was 70 per cent, while the 1943 figures yielded 153 per cent and 91 per cent, respectively. When the 1945 series, from which our present factor of 3.6 was derived, was subjected to statistical analysis, a coefficient of variation of 55 per cent was obtained.

DISCUSSION AND CONCLUSIONS

Considerable criticism has been leveled from time to time at the procedure of pollen counting. The clinician knows that there is fair correlation between pollen concentration and clinical symptoms, even though the counts do not accurately or constantly bear this out. Furthermore, he is aware that extra pollen factors, both allergic and nonspecific, modify the course and severity of clinical hay fever. Much of the blame for this divergence must be attributed to our neglect of the physical laws governing the distribution of particulate substances in the air and to our inability to devise a perfect atmospheric sampler. Another source of error lies in our attempt to convert gravimetric surface area counts to volumetric equivalents. These experiments have shown how inconsistent the ratios are from year to year. Attention has also been called to previous experiments by other investigators demonstrating similar variability. Since the total daily pollen exposure is obtained by multiplying the pollen per cubic yard of air by the volume of air inhaled, it is easy to see how a variable conversion factor would give incorrect data. Allergists, confused by figures that did not correlate with their clinical observations, developed a healthy skepticism as to its value. Obviously, the only meaningful figures are the volumetric ones. Such figures can be obtained only with a standard volumetric sampling device, as originally recommended by the National Pollen Survey Committee. With such a device, it would be possible to obtain more accurate pollen counts that should correlate better with clinical symptoms. Such an apparatus could also serve as a research tool in determining the various states that affect pollen liberation and govern its distribution. Clinically, it could be used to test the efficiency of the various air-filtering, air-processing, and air-conditioning apparatuses that are marketed for this purpose. Information of this sort would be invaluable to the clinician in advising his patients of the relative proficiency of air-processing equipment.

These experiments have served to emphasize the shortcomings of our present method of air sampling, particularly in reference to converting surface area counts to their volumetric equivalents. Considerable variability of the daily and annual volumetric-gravimetric ratios was demonstrated. Until a satisfactory standard volumetric apparatus is accepted for general use, it is suggested that all gravity slide counts be recorded as pollen granules per square centimeter of slide area and that no attempt be made to translate such figures into volumetric equivalents by the use of conversion factors.

The statistical analysis in this study was made by Samuel Askovitz, M.D.

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2039 DELANCY PL. (G.I.B.)

Erratum

In the article entitled "High-Efficiency Pollen Samplers for Use in Clinical Allergy" by Harrington, Gill, and Warr, which begins on page 357 of the July—August, 1959, issue of the JOURNAL, the equations on page 361 should read as follows:

$$K = \frac{2}{9} \frac{\rho p \, r^{2u}}{\mu L} \quad \text{and} \quad \emptyset = \frac{18 \, \rho a^2 \, LU}{\mu \rho p}$$

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TO THE EDITOR:

In the November—December, 1958, issue of THE JOURNAL OF ALLERGY (volume 29, No. 6, pages 542-544) we reported a case of hypersensitivity following ingestion of hydroxyzine (Atarax) in a meprobamate-sensitive patient.

In our comment we stated: "In view of the reports of the almost complete absence of toxic or hypersensitive reaction to hydroxyzine, it is with some trepidation that we present this case which suggests such probability. The possibility that this patient's anaphylactic-like reaction following the ingestion of hydroxyzine represented a delayed serum type of reaction to a meprobamate hapten does not seem likely. The possibility that meprobamate, although not chemically closely related, could sensitize a patient to hydroxyzine is to be considered. The desirability that this patient again be given hydroxyzine to determine whether or not sensitivity to the drug is present is recognized. So far we have been unable to gain his consent to take even a small amount of the drug under controlled conditions."

This patient agreed to enter the Norton Memorial Hospital on March 13, 1959, and consented to take the Atarax again. He was given a 10 mg. capsule with no untoward effect, and this was then repeated three times daily. On discharge (March 15, 1959), he was given a prescription of 25 mg. Atarax capsules. The patient continued to take the Atarax without untoward result.

In view of the fact that this patient is able to take Atarax with no apparent deleterious effect, it must be concluded that the hypersensitive reaction previously reported was probably due to the delayed reaction of meprobamate.

It is regretted that our previous article suggested that this patient's allergic reaction was due to hydroxyzine (Atarax).

Very truly yours,

ARMAND E. COHEN, M.D.

June 29, 1959.

OBITUARIES

CLARENCE BERNSTEIN

CLARENCE BERNSTEIN died at his home in Orlando, Florida, on April 23, 1959, after a long illness due to bronchial adenomatosis.

Dr. Bernstein was born in Nashville, Tennessee, on July 19, 1905. He received his A.B. degree from Yale University in 1926 and his M.D. degree from Johns Hopkins University Medical School in 1931; he was elected to both Phi Beta Kappa and Alpha Omega Alpha. From 1928 to 1932 he participated in investigative work in pathology and immunology at Johns Hopkins, receiving the Mellon Research Fellowship for the year 1931-1932. He served as house officer on the Harvard Medical Service at Boston City Hospital in 1932 and 1933.

Dr. Bernstein was assistant in the Department of Medicine at the University of Chicago from 1933 to 1935, where he was elected to membership in Sigma Xi. He also engaged in private medical practice in Chicago and served on the staffs of the Michael Reese and Cook County Hospitals. He was the consultant for the Institute of Psychoanalysis of Chicago and became a member of the faculty at Northwestern University Medical School. He was a member of the Chicago Allergy Society, the Institute of Medicine of Chicago, and the Central Society for Clinical Research. He was certified in internal medicine in 1939 and in allergy in 1944.

Because of his pulmonary symptoms, in 1943 Dr. Bernstein moved to Florida, where he entered private practice in Orlando, later becoming associated with Drs. S. D. Klotz and Albert M. Ziffer. He was a member of the staffs of Orange Memorial Hospital, Florida Sanitarium and Hospital, and the Winter Park Memorial Hospital.

Dr. Bernstein was elected to membership in the American Academy of Allergy in 1944 and was advanced to Fellowship in 1946; he served on the Executive Committee from 1955 to 1958. He was a Fellow of the American College of Allergists and was program chairman of the Session on Allergy, American Medical Association, 1957. He was a member and past president of the Southeastern Allergy Society, a founder and past president of the Florida Allergy Society, and an honorary member of the Cuban Allergy Society. Other professional memberships included the American Psychosomatic Society, the American Federation of Clinical Research, the American

Association for the Advancement of Science, the American Medical Association, the World Medical Association, and the Federation of American Scientists. He contributed some twenty-seven articles to medical and other scientific journals. He was also active in civic and religious circles.

He is survived by his wife, Babette, and daughters, Jill and Toni.

MAURICE SIMPSON FOX

MAURICE S. FOX died in Vincennes, Indiana, on Nov. 21, 1958, of coronary occlusion.

Dr. Fox was born in Freelandville, Indiana, on Feb. 4, 1901. He received his B.S. degree from Indiana University in 1928 and his M.D. degree in 1930, after which he entered practice with his father, Dr. Richard Fox, at Freelandville, Indiana. In 1943, after retirement from the army following an attack of coronary occlusion, Dr. Fox took training in allergy under Dr. O. E. Egbert of El Paso, Texas, and in 1945 he limited his practice to allergy in Vincennes, Indiana.

Dr. Fox was elected to membership in the American Academy of Allergy in 1946. He was a Fellow of the American College of Allergists and served on the staffs of the Good Samaritan Hospital and the Lawrence County Memorial Hospital. He was also a veteran of World War II.

On June 10, 1931, Dr. Fox married Esther Keeling, R.N. Mrs. Fox and their daughter, Susan Jane, and son, Richard, survive him.

Mark H. Mothersill.

JEROME FRANKEL

JEROME FRANKEL died on June 1, 1958.

Dr. Frankel was born in Pultusk, Poland, on April 15, 1913, and came to the United States in 1922. In 1935 he received his A.B. degree at Western Reserve University, and in 1936 he received the degree of M. Sc. at Ohio State University. His M.D. and Ph.D. degrees were conferred by Ohio State University in 1941. Following discharge from the army with the rank of major in 1946, he served a residency in medicine at Mount Sinai Hospital, Cleveland, Ohio, after which he entered practice in Cleveland. In 1949 he was appointed demonstrator in medicine at Western Reserve University School of Medicine and became a member of the staff of Mount Sinai Hospital, where he served as physician in charge of the outpatient department, head of the Personnel Health Clinic, and secretary of the Medical Education Committee.

Dr. Frankel was elected to Fellowship in the American Academy of Allergy in 1957. He also was a member of the American Medical Association, the Ohio State Medical Association, the Academy of Medicine of Cleveland, the Cleveland Allergy Society, and the American College of Allergists. He

was also a founding member of the American Board of Clinical Chemistry. He was a diplomate of the American Board of Internal Medicine and a Fellow of the American College of Physicians.

Dr. Frankel is survived by his wife, Mrs. Anna R. Frankel, two sons, and one daughter. To them his colleagues in the Academy of Allergy extend their most sincere sympathy.

Myron A. Weitz.

BARNETT P. FREEDMAN

BARNETT P. FREEDMAN of New Haven, Connecticut, died at his home on Tumblebrook Road in Woodbridge, Connecticut, on Tuesday, April 14, 1959.

Dr. Freedman was born in New Haven on Sept. 6, 1896. After graduating from Yale Sheffield Scientific School in 1916, he attended Yale University School of Medicine, receiving his M.D. degree in 1920. He served his internship and residency at the City Hospital of New York. After several years in general medicine, his interests led him into the field of allergy; he was one of the first allergists in New Haven. He became associated with the Allergy Department of the Brooklyn Jewish Hospital, where he served diligently and faithfully for over twenty years. Dr. Freedman was a consultant in allergy at the Grace New Haven Hospital for a number of years.

"Barney," as we all called him, was a founder and the second president of the Connecticut State Allergy Society. He was a member of the American Academy of Allergy, the American Medical Association, the New Haven County Medical Society, and the Connecticut State Medical Society. Although a specialist in allergy for many years, he never lost the sympathetic interest and the informal, friendly approach of the family practitioner. The devotion and love that his patients held for him was in direct proportion to the service and personal interest he devoted to them.

Barney was as modest and unassuming a person as I have ever known. He was marked with kindness, gentleness, humility, and a sense of humanity that endeared him to all. He never sought fame or glory, and his bigness was his integrity and the manner in which he lived his life. We, who were close to him, will miss his graciousness, his pleasant manner, and his fair, unjealous disposition.

Dr. Freedman is survived by his wife, Lillian, and by two sons, Jerry (a physician) and Arthur.

Sidney W. Jennes.

ALEXANDER SOCHACKI

ALEXANDER SOCHACKI of Camden, New Jersey, died on July 26, 1958.

Dr. Sochacki was born in 1903. He obtained his M.D. degree from the University of Pennsylvania in 1928. He was chief of the allergy service, Cooper

Hospital, Camden, New Jersey; assistant in allergy, Jefferson Hospital, Philadelphia; and demonstrator in allergy, Jefferson Medical School.

He was elected to membership in the American Academy of Allergy in 1946. Other affiliations included the American College of Allergists, the Philadelphia Allergy Society, the International Correspondence Society of Allergists, and the World Medical Association.

Homer E. Prince.

NATHAN SWERN

NATHAN SWERN of Trenton, New Jersey, died on July 27, 1958.

Dr. Swern was born in 1897. He received his M.D. degree from Jefferson Medical College in 1922. He was certified in medicine and in allergy. Hospital and teaching appointments included attending physician and consultant in metabolism, St. Francis Hospital; consulting physician, Orthopedic Hospital; clinical assistant in allergy, Jefferson Hospital; and assistant in medicine, Jefferson Medical College.

Dr. Swern was elected to membership in the American Academy of Allergy in 1939 and was advanced to Fellowship in 1947. He was also a Fellow of the American College of Allergists, the American College of Physicians, the American Dermatological Association, the New Jersey Allergy Society, the Philadelphia Allergy Society, and the New Jersey Diabetes Association. He served in World War I.

Homer E. Prince.

ANNOUNCEMENTS

Allergy Foundation of America

National Allergy Month, August 15—September 15.—National Allergy Month is an intensive campaign of public information and education conducted through the press and over radio and television by the Allergy Foundation of America, with the support of the Advertising Council, Inc. It is intended to increase public awareness and understanding of allergy and the allergic diseases. It has been held from August 15 to September 15 each year since 1957 and is timed to coincide with the peak of the ragweed hay fever season in a large portion of the United States.

This year, as in previous years, the Foundation has prepared a special free pamphlet for distribution to the public. It is entitled *Asthma, Hay Fever and Other Allergies,* reflecting the fact that the pamphlet contains more information on asthma and its treatment and new material on allergy in children and food allergies. The public may obtain single copies by writing to P. O. Box 1005, New York 17, New York. Physicians who wish to distribute the pamphlet to their patients may obtain them in quantity from the Foundation at no cost other than the shipping charge.

Mr. Russell Ziegler Elected President.—Mr. Russell Ziegler of New York, Director of Advertising and Public Relations for Cluett, Peabody and Company, Inc., was elected president of the Allergy Foundation of America at the annual meeting of the Foundation's Board of Trustees on June 12, 1959.

Mr. Ziegler succeeds Mr. John D. Butt, recently retired chairman of the Board and former president of The Seamen's Bank for Savings, who, until his resignation, had served the Foundation first as treasurer and subsequently as president. Mr. Butt will remain a member of the Foundation's Board of Trustees and its Executive Committee.

At the same meeting, Dr. Horace S. Baldwin of New York City, a former president of the American Academy of Allergy and a founding member and first president of the Foundation, was re-elected chairman of the Board of Trustees. Other officers elected for the next year are: *Vice-Presidents*—Mr. Wyeth Allen of Ann Arbor, Michigan, Mr. Oscar Chapman of Washington, D. C., and Dr. John D. Gillaspie of Boulder, Colorado; *Secretary*—Dr. M. Murray Peshkin of New York City; and *Treasurer*—Mr. Roderick McRae of New York City.

Newly elected members of the Board of Trustees include Dr. Giles A. Koelsche of Rochester, Minnesota, Dr. Bram Rose of Montreal, Canada, and Mr. Louis Bielfield of Detroit, Michigan.

Foundation Cites Dr. Jules Freund.—The Allergy Foundation of America has presented a citation to Dr. Jules Freund, who is in charge of basic studies on allergy and immunology at the National Institute of Allergy and Infectious Diseases, honoring him on his sixty-ninth birthday for his many contributions to medical science.

Dr. Freund's work in preventive medicine, experimental pathology, and immunology has been largely in basic research. His studies of antibody formation, toxin-antitoxin reactions, and demyelination processes have provided important new fundamental knowledge. A most valuable contribution was his demonstration of the increased effectiveness of vaccines in which the protective materials are incorporated in water-in-oil emulsions. The latter, known as Freund's adjuvant, is used in basic research by immunologists everywhere.

Active also as a teacher, lecturer, consultant, writer, and editor, Dr. Freund is recognized the world over as an outstanding investigator in immunology. A past president of the American Association of Immunologists and recent chairman of the Board of the Federation of American Societies for Experimental Biology, Dr. Freund has also served as a member of the editorial board of the *Journal of Immunology* and as a consultant to the Department of the Army and the Public Health Service.

Dr. Freund came to the United States from his native Hungary in 1923 to continue his career of research and teaching in Philadelphia and New York. Before joining the NIH, he was chief of the Division of Applied Immunology, Public Health Research Institute of the City of New York.

New Exhibit for Lay Audiences.—The Foundation has prepared a simple, lightweight exhibit for showing to lay audiences—at fairs, meetings, in window displays, etc. Intended for table-top use (though it can be used at floor level), this colorful and eye-catching exhibit measures eight feet in over-all length and three and one-half feet in height. It consists of three panels which interdigitate with four sturdy wooden supports. Shipped in a fiberboard container, secured by webbed straps, the weight is less than 40 pounds. One person can erect or dismantle the exhibit; it can be carried for short distances with ease.

The message is simple. One side panel lists the more common allergic diseases, and the other lists the objectives of the Foundation. The central panel displays the printed materials available from the Foundation for public information and education. Any or all of the Foundation's *free* pamphlets are available in quantity for distribution with the exhibit.

The exhibit is available for the cost of shipping plus a $10.00 service charge. Pamphlets are provided for the cost of shipping.

Seventh Congress of the German Society of Allergology

At the seventh congress of the German Society of Allergology, which will be held Oct. 8 to 10, 1959, in Bad Lippspringe, West Germany, the following guest speakers will read their reports:

Symposium I (Allergy to House Dust and Moulds)

R. Voorhorst, Leiden, Netherlands.
P. J. van der Werff, Amersfort, Netherlands.

Symposium II (Hyposensitization—Theoretical and Practical Aspects)

F. Scheiffarth, Erlangen, Germany.
H. Schmidt, Freiburg, Germany.
W. Gronemeyer, Bad Lippspringe, Germany.
K. Hajós, Budapest, Hungary.
D. Zerykier, Tel-Aviv, Israel.

In addition to this program, numerous sectional papers will be presented.

Further information will be given by the Organizing Committee of the Congress, c/o Kurverwaltung Bad Lippspringe, West Germany.